UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

Date: March 29, 2016

SUBJECT: Dicamba. Section 3 Registration for the Amended Use of Dicamba on Dicamba-Tolerant Cotton. Summary of Analytical Chemistry and Residue Data.

PC Code: 029801, 029802, 029806, 128931, 128944, & 129043	**DP Barcode:** D408384
Decision No.: 467997	**Registration No.:** 524-582
Petition No.: 2F8067	**Regulatory Action:** Amended Section 3 Registration
Risk Assessment Type: NA	**Case No.:** 0065
TXR No.: NA	**CAS No.:** 1918-00-9
MRID Nos.: 48728701-48728704	**40 CFR:** §180.227

FROM: Peter Savoia, Chemist
Risk Assessment Branch V/VII
Health Effects Division (7509P)

THRU: Michael S. Metzger, Branch Chief
Donna S. Davis, Chemist
Risk Assessment Branch V/VII
Health Effects Division (7509P)

TO: William A. Irwin, Risk Assessor
Risk Assessment Branch V/VII
Health Effects Division (7509P)
And
Kathryn Montague/Michael Walsh, Risk Manager
Herbicide Branch
Registration Division (7505P)

Executive Summary

Monsanto has submitted petition PP#2F8067 requesting Section 3 registration for the amended use of dicamba on dicamba-tolerant cotton. Monsanto is requesting to amend the cotton undelinted seed tolerance from 0.2 ppm to 3.0 ppm and establish a new tolerance for cotton gin byproducts at 70.0 ppm. Dicamba is a selective benzoic acid herbicide used for controlling weeds prior to their emergence. It is available for use in either acid or salt forms with registered uses being maintained on a wide variety of crop and livestock commodities. Permanent tolerances are established under 40 CFR §180.227(a)(1) for dicamba and its 3,6-dichloro-5-hydroxybenzoic acid (5-OH dicamba) metabolite. Additional tolerances are established under 40 CFR §180.227(a)(2) for dicamba and its 3,6-dichloro-2-hydroxybenzoic acid (also known as 3,6-diclorosalicyclic acid or DCSA) metabolite, as well as under 40 CFR §180.227(a)(3) for dicamba, 5-OH dicamba, and the DCSA metabolite.

Dicamba is registered for pre-plant application on cotton but not for post-emergence treatment because crop injury could occur if it were to come in contact with cotton roots, stems, or foliage. To enable post-emergence application, Monsanto has developed a dicamba-tolerant variety of cotton (MON 88701) capable of receiving treatment up to seven days before harvest. The M1691 Herbicide (EPA Reg. No. 524-582) soluble liquid concentrate (SL) diglycolamine salt formulation of dicamba is proposed for use on dicamba-tolerant cotton. For pre-emergent application, up to 32 fluid ounces of the M1691 herbicide (1.0 lb ae/A) may be used for pre-plant or at planting prior to crop emergence. Using a maximum single in crop application of up to 16 fluid ounces per acre (0.5 lb ae/A), post-emergence treatments are also allowed not to exceed the seasonal total in a given year. Applications are to be made following a 7-day re-treatment interval (RTI) and a 7-day pre-harvest interval (PHI). A combined total of all applications is not to exceed 64 fluid ounces per acre (2.0 lb ae/A) in a given year.

Previously submitted wheat, grape, asparagus, sugarcane, cotton, and soybean metabolism studies demonstrate that dicamba is metabolized in plants by demethylation and hydroxylation. A new metabolism study on dicamba-tolerant cotton shows a generally similar metabolic pathway to other plants; however, when compared to cotton which is not dicamba-tolerant, significantly more DCSA is found with the dicamba-tolerant cotton. Therefore, the newly submitted metabolism study supports including parent, 5-OH dicamba, as well as the DCSA metabolite as the residues of concern for dicamba-resistant cotton for both tolerance setting purposes and risk assessment. Based on previously submitted metabolism studies on ruminants and poultry, the nature of the residue in livestock commodities for tolerance setting purposes and risk assessment is adequately understood to be dicamba and DCSA.

There are adequate methods available for the enforcement of the newly proposed cotton seed and cotton gin byproducts tolerances. PAM Vol. II Method I using Gas Chromatography/Electron Capture Detection (GC/ECD) analyses is adequate for enforcement. New tolerances are not proposed for animal commodities; therefore a discussion of animal enforcement methods is not relevant to this petition. Dicamba is recoverable through the FDA multi-residue method (MRM) testing protocols, but no data have been provided for any of its regulated metabolites. However, single analyte methods are available for enforcement and additional MRM data are not needed.

The number and locations of the field trial studies submitted for dicamba-resistant cotton were in accordance with the OCSPP 860.1500 test guidelines. The data were generated using a validated data collection method and are supported by adequate storage stability data. Total residues of dicamba and its metabolites 5-OH dicamba, and DCSA, expressed as parent equivalents, ranged from 0.11 ppm to 1.72 ppm in dicamba-resistant cotton undelinted seed treated at a maximum seasonal application rate of 2.0 lbs ae/A with a single late-season foliar application of 0.5 lbs ae/A and a PHI of 7-days. For gin byproducts in dicamba-tolerant cotton, total residues of dicamba ranged from 5.06 ppm to 29.6 ppm following this same pattern of use.

An adequate processing study was submitted. Residue data was generated using an adequately validated data collection method. Samples were analyzed within 30-days; therefore, storage stability data is not required. Residues of dicamba, 5-OH dicamba, and DCSA did not concentrate in any processed fraction.

The residue values obtained from these field trial studies and the processing study were evaluated using the Organization for Economic Cooperation and Development (OECD) calculation procedures

for estimating tolerances/Maximum Residue Limits (MRLs). Using the OECD calculation procedures, and inputting the total residue, which includes the parent compound, and its metabolites 5-OH dicamba, and DCSA, expressed as parent, recommended tolerances of 3.0 ppm for cotton undelinted seed and 70.0 ppm for cotton gin byproducts are appropriate.

For this action, the establishment of a tolerance on cotton gin byproducts will not increase livestock dietary burden; therefore, no new revised tolerances on livestock commodities are required to support this petition.

There are MRLs of 0.2 ppm in Mexico and 0.04 ppm established by Codex on cotton seed currently established. There are currently no Mexican, Canadian or Codex MRLs established for cotton gin byproducts. Since the registrant has requested a late season use of dicamba on dicamba-tolerant cotton, the currently established international tolerances are not adequate to cover residues likely from the newly proposed use in the U.S. In addition, the dicamba residues of concern for dicamba-tolerant cotton also include the DCSA metabolite which is not found nor regulated in the other common varieties of cotton. Therefore, harmonization is not possible at this time for cotton seed. Since there are no international tolerances on cotton gin byproducts, there is no issue of international harmonization relevant to that tolerance.

The December 2005 Reregistration Eligibility Decision (RED) for dicamba requires additional rotational crop studies in order to satisfy data requirements. However, these data are not needed if a 120-day plantback interval is specified when dicamba is applied at the maximum seasonal rate of 0.75 lb ae/A. For greater seasonal application rates of 0.75-2.0 lb ae/A, only crops with established tolerances can be rotated for planting. No new rotational crop data have since been submitted; therefore, the plantback restrictions noted in the 2005 RED are appropriately specified on the product label for treating dicamba-tolerant cotton; and additional rotational crop data are not needed at this time.

Regulatory Recommendations and Residue Chemistry Deficiencies

Provided the label is amended as specified below, there are no residue chemistry deficiencies that preclude establishing permanent tolerances on cotton raw agricultural commodities (RACs) for dicamba. A revised human health risk assessment to support the requested use on dicamba-resistant cotton is forthcoming.

The tolerance expressions specified in the CFR for dicamba are in compliance with HED's Interim Guidance on Tolerance Expressions (05/27/2009, S.Knizner). For this action, the recommended dicamba tolerances for cotton RACs are to be set under 40 CFR §180.227(a)(3) as follows:

Tolerances are established for residues of the herbicide dicamba, 3,6-dichloro-o-anisic acid, including its metabolites and degradates, in or on the commodities in the table below. Compliance with the tolerance levels is to be determined by measuring only the residues of dicamba, 3,6-dichloro-o-anisic acid, and its metabolites, 3,6-dichloro-5-hydroxy-o-anisic acid, and 3,6-dichloro-2-hydroxybenzoic acid, calculated as the stoichiometric equivalent of dicamba, in or on the following commodities:

Cotton, gin byproducts	70.0 ppm
Cotton, undelinted seed	3.0 ppm

Note to RD

The 40 CFR §180.227(a)(1) citation for dicamba currently lists a 0.2 ppm tolerance for cotton, undelinted seed. This entry in the CFR should be removed, and replaced with the tolerances shown above under 40 CFR §180.227(a)(3).

860.1200 Directions for Use

An amended Section B is required noting that no more than two (2) post-emergence applications may be made past the first open boll stage when treating dicamba-tolerant cotton is allowed.

Background

Dicamba, 3,6-dichloro-2-methoxybenzoic acid, is a selective benzoic acid herbicide registered for controlling a wide variety of broadleaf weeds and woody plants prior to their emergence. It has similar hormonal properties to natural auxins which induce abnormal and uncontrollable growth to disrupt normal plant functions at high concentrations. Dicamba end-use products are available as either acid or salt formulations with registered uses being maintained on a wide variety of crop and livestock commodities. The chemical structure and nomenclature of dicamba and its metabolites 5-OH dicamba, and DSCA are presented in Table 1. The physicochemical properties of the technical grade of dicamba acid are presented in Table 2.

Table 1. Test Compound Nomenclature: Dicamba and its Residues of Concerm.

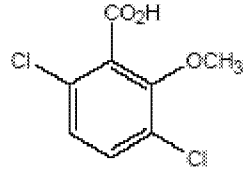
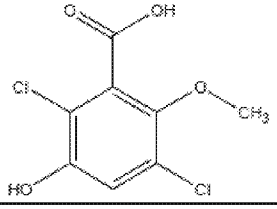
Compound	
Common name	Dicamba
Company experimental name	MON 11900
IUPAC name	3,6-dichloro-o-anisic acid or 3,6-dichloro-2-methoxybenzoic acid
CAS name	3,6-dichloro-2-methoxybenzoic acid
CAS registry number	1918-00-9 (dicamba acid), 104040-79-1 (diglycolamine salt), or 53404-28-7 (monoethanolamine salt)
End-use product	Clarity® Herbicide: SL formulation containing 4 lb ae/gal
Compound	
Common name	5-Hydroxy-dicamba
Company experimental name	5-OH dicamba
IUPAC/CAS name	2,5-dichloro-3-hydroxy-6-methoxybenzoic acid
CAS registry number	7600-50-2

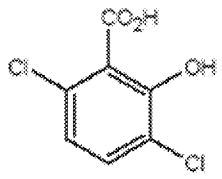
Table 1. Test Compound Nomenclature: Dicamba and its Residues of Concerm.	
Compound	
Common name	DCSA; 3,6-dichlorosalicylic acid
Company experimental name	MON 52708
IUPAC/CAS name	3,6-dichloro-2-hydroxybenzoic acid
CAS registry number	3401-80-7

Table 2. Physicochemical Properties of the Technical Grade Test Compound: Dicamba.		
Parameter	Value	Reference
Melting point	114-116 EC (PAI) 90-100 EC (87% TGAI)	Residue Chemistry Chapter of the Dicamba RED (D317699, C. L. Olinger, 12/20/2005).
pH	2.5-3.0 (87% TGAI)	
Density	1.57 g/mL at 25 EC (87% TGAI)	
Water solubility	0.5 g/100 mL at 25 EC (PAI)	
Solvent solubility	g/100 mL at 25 EC (PAI) dioxane 118.0 ethanol 92.2 isopropyl alcohol 76.0 methylene chloride 26.0 acetone 17.0 toluene 13.0 xylene 7.8 heavy aromatic naphthalene 5.2	
Vapor pressure	3.4 x 10^{-5} mm Hg at 25 EC (PAI)	
Dissociation constant, pK_a	1.97 (PAI)	
Octanol/water partition coefficient, Log(K_{OW})	0.1 (PAI)	
UV/visible absorption spectrum	neutral: 511 (275 nm) acidic (pH 0-1): 1053 (281 nm) basic (pH 13-14): 469 (274 nm)	

860.1200 Directions for Use

The dicamba product used for treating dicamba-tolerant cotton proposed for registration is the M1691 Herbicide (EPA Reg. No. 524-582) which is a soluble liquid concentrate (SL) formulation. This end–use product contains 56.8% active ingredient in the form of the diglycolamine salt of dicamba (equivalent to 4.0 lb ae/gal). A summary of the proposed directions for use taken directly from the supplemental M1691 herbicide label provided by the registrant are presented below in Table.

Table 3. Summary of Directions for Use of Dicamba.								
Formulation [EPA Reg. No.]	Applic. Timing, Type, and Equip.	Max. Applic. Rate (lb ae[1]/A)	Max. No. Applic. per Season	Max. Seasonal Applic. Rate (lb ae/A)	Combined Max. Seasonal Applic. Rate (lb ae/A)	RTI[2] (days)	PHI[3] (days)	Use Directions and Limitations[4]
MON 88701 Cotton								
M1691 4.0 lb ae/gal SL [524-582]	Pre-plant, at planting, and Pre-emergence Broadcast (20 gal/A)	1.0	NS[5]	1.0	2.0	7	7	Use of a COC or MSO is not recommended with Roundup branded herbicides. These adjuvants are only used when other products require them. For best results apply at min spray rate of 10 GPA. Apply with ground equipment only; aerial application is prohibited.
	Post-emergence, Broadcast (20 gal/A)	0.5	NS	2.0				

[1] ae = Acid Equivalents
[2] RTI = Re-Treatment Interval
[3] PHI = Pre-Harvest Interval
[4] COC = Crop Oil Concentrate; MSO = Methylated Seed Oil.
[5] NS = Not Specified

Conclusions. The proposed directions for use were compared to the submitted field trial data. The field trial studies were conducted covering the expected range of applications allowed under the proposed label. This includes pre-emergent applications of up to 1.0 lb ae/A, as well as multiple early to late post-emergent treatments of up to 0.5 lb ae/A each for a combined annual total of 2.0 lb ae/A. Treatments may include the use of adjuvants and can be re-applied following a 7-day RTI and a 7-day PHI. As such, the product label allows growers great flexibility in using dicamba to control weeds when cultivating cotton. The residue data provided examining this broad pattern of use shows that later post-emergence treatments give much higher residues then those made at earlier growth stages. Following the pattern of late season use demonstrated by the field trial data, no more than two (2) post-emergence applications may be made after the first open boll stage when treating dicamba-tolerant cotton. Therefore, the registrant should amend the product label to include this restriction since the data provided only support this pattern of use.

860.1300 Nature of the Residue - Plants

Reregistration Eligibility Decision Memo, D317699, 12/20/2005, C. L. Olinger

DER Reference: 48728701.der.docx (Nature of the Residue in Dicamba-Tolerant Cotton)

For non-dicamba resistant plants, the nature of the residue was previously determined to be understood (D317699, C. L. Olinger, 12/20/2005). Prior plant metabolism studies were reviewed in part with the 1983 residue chemistry chapter of the dicamba registration standard. The studies

demonstrate that dicamba is rapidly absorbed and translocated by grasses, grapes, black valentine beans, wheat and bluegrass, as well as in soybeans. Dicamba is metabolized in plants mainly by demethylation and hydroxylation.

The registrant has developed a dicamba-tolerant cotton (known as MON88701), which contains a gene that expresses an enzyme to catalyze the breakdown of dicamba. This enzyme, O-demethylase, works by cleaving a specific carbon-oxygen bond converting the aromatic methoxyl group of dicamba into an aromatic hydroxyl group to form the non-herbicidal DSCA metabolite. DSCA is a known metabolite of dicamba found in soil, plants, and livestock. The petitioner has submitted a new metabolism study for dicamba-tolerant (MON 88701) cotton (48728701.der, P. Savoia, 03/25/2013). A summary of the characterization and identification of residues is shown in Appendix I along with the chemical names and structures of the identified metabolites illustrated in Appendix II. A summary of the data and findings follow.

For this study, metabolism was found to occur at an appreciable rate with the first step in the process being demethylation of parent into the DCSA metabolite. Residue levels were considerably lower for the pre-emergence samples in comparison to those obtained following post-emergence treatment. Greater residue concentrations were obtained for the cotton leaves/stems (surrogate gin byproducts) as opposed to seed. These data indicate that residues tend to be on the surface of the dicamba-tolerant cotton plant. Parent dicamba was identified in all the matrices tested which include both gin byproducts and in seed at lower levels. Its metabolites DCSA glucoside, DCSA, and DCGA glucoside were found to be present in all matrices as well. The 5-OH dicamba metabolite was not identified in any matrix. For dicamba-tolerant cotton, DCSA glucoside was the major metabolite obtained with the highest levels being found in the gin byproducts samples.

Based on these results, the petitioner proposes that the metabolism of dicamba in MON 88701 cotton proceeds by the demethylation of dicamba to form the DCSA metabolite. The formation of DCSA occurs through the action of the dicamba *O*-demethylase enzyme produced by the dicamba mono-oxygenase gene introduced into this variety of cotton for tolerating application of the herbicide. The petitioner notes that due to the predominance of the demethylation pathway, 5-OH dicamba is not observed as a metabolite found in either dicamba-tolerant cotton or soybean.

In consultation with the HED Residues of Concern Knowledgebase Subcommittee (ROCKS) co-chairs on March 18, 2013, the residues of concern (ROC) in dicamba-tolerant cotton were determined for both tolerance setting and risk assessment purposes. The newly submitted data supports including parent and the DCSA metabolite, as well as the 5-OH dicamba metabolite found in non-resistant varieties as the residues of concern in cotton for tolerance expression and risk assessment.

The rationale for the ROC decision is as follows. Residues present in both cotton and dicamba-tolerant cotton were comprised of dicamba, and its metabolites, 5-OH dicamba, DCSA, and DCGA. While the DCGA metabolite was detected in field trial samples, and can be considered of comparable toxicity to the parent compound, the available analytical enforcement methodology is not considered validated for the DCGA metabolite. However, dicamba, 5-OH dicamba, and DCSA account for the majority of the residues in both tolerant and non-tolerant cotton and will provide sufficient residues with which to monitor for misuse for both tolerant and non-tolerant cotton; therefore, the ROC for tolerance setting purposes is dicamba, 5-OH dicamba, and DCSA.

For the purpose of risk assessment, HED considered both the exposure and hazard profiles for dicamba, 5-OH dicamba, DCSA, and DCGA and is including dicamba, 5-OH dicamba, and DCSA as the ROC for tolerant and non-tolerant cotton. Based on available toxicity studies and structural similarities, HED considers the parent and all three metabolites to be of comparable toxicity. While DCGA may be of comparable toxicity, it was present in the cotton metabolism studies at less than 10% of the total radioactive residue (TRR), and is only detected in livestock feed items, not in human food items. Further, inclusion of this metabolite as a ROC in feed items would have no material impact on the livestock dietary burden since calculation of the reasonably balanced livestock diets are driven by other feed items with far higher residues. Thererfore, HED is not including the DCGA metabolite as an ROC for risk assessment. The residues of concern that were concluded for tolerance expression and risk assessment in crop commodities based on these data are presented below in Table 4.

Table 4. Dicamba Residues of Concern.

Matrix	Tolerance Expression	Residues for Risk Assessment
Barley, corn, grasses, oats, proso millet, sorghum, sugarcane, and wheat	Dicamba + 5-OH dicamba	Dicamba + 5-OH dicamba
Asparagus	Dicamba + DCSA[1]	Dicamba + DCSA
Cotton, Soybeans and aspirated grain fractions (AGFs)	Dicamba + 5-OH dicamba + DCSA	Dicamba + 5-OH dicamba + DCSA

[1] DCSA also referred to as 3,6-dichloro-2-hydroxybenzoic acid or as 3,6-dichlorosalicylic acid.

860.1300 Nature of the Residue - Livestock

Reregistration Eligibility Decision Memo, D317699, 12/20/2005, C. L. Olinger

The nature of the residue in livestock is adequately understood based upon acceptable metabolism studies made on ruminants and poultry. The residues of concern in meat, milk poultry, and eggs are dicamba and its DCSA metabolite established under 40 CFR §180.227(a)(2). A new livestock commodity storage stability study and ruminant feeding study made at an increased dicamba parent feeding level of 1000 ppm was provided in support of reregistration. Both studies were determined to be adequate and no further livestock storage stability or ruminant feeding studies are required (D320564 & D322482, C. L. Olinger, 12/08/2005).

860.1340 Residue Analytical Methods

Plant commodities

Reregistration Eligibility Decision Memo, D317699, 12/20/2005, C. L. Olinger

DER Reference: 48728702.der.docx (Residue Analytical Method for Dicamba-Tolerant Cotton)

Enforcement method: Adequate methods available for the enforcement of the newly proposed cotton seed and cotton gin byproducts tolerances. The Pesticide Analytical Manual (PAM) Vol. II lists Method I (AM 02658A) which uses GC/ECD analysis to carry out the determination of dicamba in/on crop commodities. The sensitivity of this method is listed to be 0.05 ppm and it has been validated by the Agency using corn and sorghum commodities. An improved plant enforcement method using GC/ECD analysis that includes an acid hydrolysis step for improved extraction efficiency has been developed as a modification to Method I. For this method, the reported limit of

quantitation (LOQ) is 0.02 ppm, and pending revisions suggested by the Agency, it will likewise be included in PAM Vol. II.

Data collection methods: The cotton field trial samples analyzed in support of this petition were made using a validated Liquid Chromatography/Mass Spectrometer/Mass Spectrometer (LC/MS/MS) analytical method. Developed by Monsanto as method AG-ME-13801-01, it is entitled "Analytical Method for the Determination of Dicamba and its Major Metabolites in Cotton Matrices by LC/MS/MS". For this method, samples are first extracted using a mixture of acetonitrile:water (40:60, v:v). An aliquot of the extract is then hydrolyzed with 1 N HCl at 95°C in an oven followed by partitioning with a mixture of ethyl acetate:isooctane (20:80), v:v). Water is then added to the organic phase and the sample is concentrated by evaporation until only the aqueous solution remains. The resulting samples are acidified and analyzed by LC/MS/MS determination. A complete description of the method and validation data determining dicamba along with its 5-OH dicamba, DSCA, and DCGA metabolites were included in this submission (MRID No. 48728702, P. Savoia, 03/25/2013). The method determined residues of parent, 5-OH dicamba, and DCSA separately. The method limit of quantitation (LOQ) was 0.02 ppm determined for each analyte in cotton undelinted seed, meal, hulls, and alkali refined oil. The LOQ was 0.04 ppm for cotton gin byproducts. The method was validated using samples of the various cotton matrices fortified with each analyte at 0.02, 0.20, and 10 ppm except for cotton gin byproducts which was fortified at 0.04, 0.40, and 10 ppm. Mean recoveries were within the acceptable range of 70% to 120% with the coefficient of variation less than 20% for each matrix tested to demonstrate the method is adequate for data collection. The tolerance is expressed as residues of dicamba and its metabolites expressed as dicamba; therefore, the residues of 5-OH dicamba, DCSA, and DCGA are converted to parent equivalents using molecular weight conversion factors of 0.933, 1.068, and 0.991, respectively.

Livestock commodities

The analysis of animal commodities is not relevant to this petition because no new livestock tolerances are being proposed for this action.

860.1360 Multiresidue Methods

Reregistration Eligibility Decision Memo, D317699, 12/20/2005, C. L. Olinger

Appendix II of PAM Volume I specifies that dicamba is completely recovered using Section 402 E2 of Protocol B but is only partially recovered using Section 402 E1 of Protocol B. There are no recovery data for MRM determining any of the dicamba metabolites of concern (5-OH dicamba and DCSA), and these data have been required. However, because single analyte methods for enforcement have thus been provided, the additional MRM data are not needed.

860.1380 Storage Stability

DER Reference: 48728704.der.docx (Stability of Dicamba Residues in Dicamba-Tolerant Cotton)

In support of this petition, Monsanto has submitted storage stability data for dicamba along with its DSCA and DCGA metabolites in/on MON 88701 cotton. Data on the stability of the 5-OH dicamba metabolite was not provided, and is not required since residues of that metabolite are not found in

cotton undelinted seed. The storage durations and conditions of the dicamba-tolerant (MON 88701) cotton samples studied are presented below in Table 5.

Table 5. Summary of Storage Conditions for Cotton Commodities.

Matrix (RAC or Extract)	Storage Temp. (°C)	Analyte	Maximum Storage Duration (Days)	Interval of Demonstrated Storage Stability
Cotton, undelinted seed	Held below freezing at the field sites and at -20°C at the laboratory.	Dicamba	13-196 days (0.4-6.4 months)	Residues of dicamba, DCGA, and DCSA are stable during freezer storage for up to 277 days (9 months) in undelinted cotton seed (MRID No. 48728704). The stability of 5-OH dicamba was not determined concurrently because it was not detected in cotton undelinted seed.
		5-OH Dicamba	13-122 days (0.4-4.0 months)	
		DCGA	13-285 days (0.4-9.4 months)	
		DCSA	13-121 days (0.4-4.0 months)	
Cotton, gin byproducts	Held below freezing at the field sites and at -20°C at the laboratory.	Dicamba	17-27 days (0.6-0.9 months)	Storage stability data are not required since these samples were analyzed within 30 days of harvest.
		5-OH Dicamba		
		DCGA		
		DCSA		
Cotton, hulls	Ambient at field sites, -12°C at processor, and -20°C at laboratory.	Dicamba	33-40 days (1.1-1.3 months)	Storage stability data are not required as these processed fractions were generally analyzed within 30 days of processing.
		5-OH Dicamba	33-34 days (1.1 months)	
		DCGA		
		DCSA		
Cotton, meal	Ambient at field sites, -12°C at processor, and -20°C at laboratory.	Dicamba	26-27 days (0.9 months)	
		5-OH Dicamba		
		DCGA		
		DCSA		
Cotton, alkali refined oil	Ambient at field sites, -12°C at processor, and -20°C at laboratory.	Dicamba	24-25 days (0.8 months)	
		5-OH Dicamba		
		DCGA		
		DCSA		
Cotton, RBD[1] oil	Ambient at field sites, -12°C at processor, and -20°C at laboratory.	Dicamba	19-20 days (0.6-0.7 months)	
		5-OH Dicamba		
		DCGA		
		DCSA		

[1] RBD – Refined Bleached Deodorized.

Conclusions. Adequate storage stability data for the residues of dicamba in/on dicamba-tolerant cotton commodities have been provided to support the studies provided in this submission.

860.1400 Water, Fish, and Irrigated Crops

This guideline requirement is not relevant to the current action.

860.1460 Food Handling

This guideline requirement is not relevant to the current action.

860.1480 Meat, Milk, Poultry, and Eggs

Reregistration Eligibility Decision Memo, D317699, 12/20/2005, C. L. Olinger

For this action, a 70.0 ppm tolerance will be established for cotton gin byproducts which is a livestock feedstuff fed to beef cattle. The dicamba maximum theoretical dietary burdens to livestock were previously calculated in the residue chemistry considerations for the 2005 RED. While HED is recommending the establishment of a 70 ppm tolerance for cotton gin byproducts, roughage, a tolerance of 250 ppm on grass hay, another livestock roughage feed item, was already included in the maximum theoretical dietary burden calculation; therefore, the addition of this new livestock tolerance will not materially impact the previously calculated dietary burden, as the calculation would default to the roughage with the highest impact, in theoretical dietary burden, but a maximum reasonably balanced dietary burden (MRBD) which considers reasonable diets for livestock, rather than assuming livestock consume any of the feed stuff with the highest residue in any quantity. If HED were to recalculate the MRBD burden for dicamba at this time, not only would the cotton gin byproducts be superseded by the grass hay, so not be materially included in the calculation, but it is also likely that the overall MRBD burden would be substantially reduced from that calculated maximum theoretically dietary burden from 2005. Tolerances in livestock commodities are based on that 2005 RED dietary burden, which is likely an overestimate of livestock exposure, and not impacted by this new feedstuff tolerance; therefore, no new animal tolerances are needed to support this new use on dicamba-tolerant cotton.

860.1500 Crop Field Trials

DER Reference: 48728703.der1.docx (Magnitude of Dicamba Residues in Dicamba-Tolerant Cotton)

Monsanto has submitted crop field trial data for dicamba on dicamba-tolerant (MON 88701) cotton. Thirteen (13) field trials were conducted during the 2010 growing season in the representative North American Free Trade Agreement (NAFTA) Growing Zones. These studies were performed in NAFTA Growing Zones 2 (GA and SC; 2 trials), 4 (AR, LA, and MO; 3 trials), 6 (TX; 1 trial), 8 (OK and TX; 5 trials), and 10 (CA; 2 trials). Each trial site consisted of one untreated and three to four treated test plots.

Each treated test plot received the maximum total seasonal application rate of 2.0-2.1 lb ae/A applied following one (1) of four (4) distinct use patterns evaluated for study. The first use pattern (TRT 1) was the cultivation of the control plot which received no application of the test substances. The second use pattern (TRT 2) was performed as three foliar broadcast applications applied at pre-emergence, the 6-leaf stage, and at 15-days past the 1st white flower. The third use pattern (TRT 3) was also made as three foliar broadcast applications applied at pre-emergence, 1st open boll, and 7-days prior to harvest. The forth use pattern (TRTs 4 & 5) was done using four post-emergence foliar broadcast applications applied at the 6-leaf stage, 15-days past the 1st white flower, 1st open boll, and 7-days prior to harvest. For this use pattern, TRT 4 was carried out using a 4.0 lb ae/gal SL diglycolamine salt product proposed for registration. TRT 5 was a bridging study made using a 5.0 lb ae/gal monoethanolamine salt formulation of dicamba for testing its equivalency to the diglycolamine salt product. A summary of the use patterns is provided below in Table 6.

Table 6. Summary of Field Trial Use Patterns for Treating Cotton with Dicamba.							
TRT	Number of Sites	EP	Growth Stages/ Target Application Rates lb ae/A (kg ae/ha)				
			Pre-emergence	6 Leaf	1st White Flower + 15 days	First Open Boll	7 Days Prior to Harvest
1	13	--	--	--	--	--	--
2	13	4 lb ae/gal SL	1.0 (1.1)	0.50 (0.56)	0.50 (0.56)	--	--
3	13	4 lb ae/gal SL	1.0 (1.1)	--	--	0.50 (0.56)	0.50 (0.56)
4	13	4 lb ae/gal SL	--	0.50 (0.56)	0.50 (0.56)	0.50 (0.56)	0.50 (0.56)
5	4	5 lb ae/gal SL	--	0.50 (0.56)	0.50 (0.56)	0.50 (0.56)	0.50 (0.56)

All treatments were made using ground equipment (backpack, hand-held or tractor-mounted sprayers) with a non-ionic surfactant and ammonium sulfate added to the spray mixture at spray volumes of 18.8-21.6 gal/A. The use patterns evaluated for study followed the maximum labeled rate with pre-emergent applications all being made at 1.0 lb ae/A. The post-emergence treatments likewise followed the label using a maximum single in crop application rate of 0.5 lb ae/A made at a 7-day RTI and a 7-day PHI. The combined total of all applications appropriately followed the maximum labeled rate not exceeding 2.0 lb ae/A or 64 fluid ounces per acre in a given year. Additional samples were also collected from two trial sites for the TRT 2 and TRT 4 study use patterns to assess residue decline.

To determine the magnitude of the residue, the cotton RACs were analyzed by LC/MS/MS analysis according to Monsanto Method AG-ME-1381-01. The reported LOQ for this LC/MS/MS method is 0.02 ppm for each analyte in cotton undelinted seed and 0.04 ppm in gin byproducts. The method was successfully validated prior to and in conjunction with the analysis of the field trial samples. It is important to note that the analytical method does not specify protocol for conversion of metabolite residues to parent equivalents. The quantifiable residues of 5-OH dicamba, DCSA, and DCGA were converted to parent equivalents using molecular weight conversion factors of 0.933, 1.068, and 0.991, respectively.

The average residues for dicamba and the 5-OH dicamba and DCSA metabolites of concern for cotton undelineted seed ranged from <0.06-1.72 ppm. Cotton gin byproducts were also harvested as stripper cotton from 3 field trials following the TRT 2 use pattern, as well as from 3 additional trials from the TRT 4 use pattern. The number of samples provided for this RAC is adequate because the data from stripper only cotton for gin byproducts generally contains much more trash than picker cotton and is worst case. For the cotton gin byproducts samples that were analyzed, average residues for dicamba, and the 5-OH dicamba and DCSA metabolites of concern ranged from 0.53-29.6 ppm. These data examine the broad pattern of use offered by the label which shows that later post-emergence treatments give much higher residues then those made at earlier growth stages. No residues of dicamba were appropriately observed above the reported LOQ of 0.02 ppm for cotton undelinted seed or 0.04 ppm in gin byproducts for any of the untreated control samples. The results of the residue decline studies performed for the TRT 2 use pattern yielded residues in cotton undelinted seed at or below the 0.02 ppm LOQ. Therefore, residue decline could not be assessed for these studies. For the residue decline studies following the TRT 4 use pattern, residues of dicamba were found to decrease in undelinted cotton seed with an increasing PHI. A summary of the residue data acquired in these studies are presented in Table 7.

Table 7. Summary of Residue Data from Cotton Crop Field Trials with Dicamba.											
Commodity	Analyte	Total App. Rate lb ae/A (kg ae/ha)	PHI (days)	Residue Levels (ppm)[1]							
				n	Sample Min.	Sample Max.	LAFT[2]	HAFT[2]	Median	Mean	Std. Dev.
TRT 2 (Applications at Preemergence, 6-leaf stage, and first white flower + 15 days; EP: Clarity)											
Undelinted Cotton seed	Dicamba	2.0 (2.2)	49-105	13	<0.02	<0.02	<0.02	<0.02	0.02	0.02	N/A
	5-OH Dicamba			13	<0.02	<0.02	<0.02	<0.02	0.02	0.02	N/A
	DCSA			13	<0.02	0.23	<0.02	0.23	0.02	0.04	0.06
	Combined Residues			**13**	**<0.06**	**<0.28**	**<0.06**	**<0.28**	**0.06**	**0.09**	**0.06**
Gin byproducts	Dicamba	2.0 (2.2)	82-84	3	<0.04	<0.04	<0.04	<0.04	0.04	0.04	N/A
	5-OH Dicamba			3	<0.04	<0.04	<0.04	<0.04	0.04	0.04	N/A
	DCSA			3	0.39	1.73	0.43	1.58	0.67	0.89	0.61
	Combined Residues			**3**	**<0.47**	**<1.82**	**<0.53**	**<1.66**	**0.75**	**0.97**	**0.61**
TRT 3 (Applications at Preemergence, first open boll stage, and 7 days prior to harvest; EP: Clarity)											
Undelinted Cotton seed	Dicamba	2.0 (2.2)	6-8	13	0.06	1.97	0.06	1.38	0.65	0.64	0.43
	5-OH Dicamba			13	<0.02	0.02	<0.02	<0.02	0.02	0.02	N/A
	DCSA			13	<0.02	0.25	<0.02	0.16	0.03	0.05	0.05
	Combined Residues			**13**	**<0.12**	**<2.24**	**<0.10**	**<1.56**	**0.71**	**0.71**	**0.48**
TRT 4 (Applications at 6-leaf, first white flower + 15 days, first open boll, and 7 days prior to harvest; EP: Clarity)											
Undelinted Cotton seed	Dicamba	2.0-2.1 (2.2-2.4)	6-8	13	0.09	1.54	0.12	1.42	0.47	0.61	0.41
	5-OH Dicamba			13	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	N/A
	DCSA			13	0.02	0.27	0.02	0.27	0.06	0.08	0.07
	Combined Residues			**13**	**<0.13**	**<1.83**	**<0.16**	**<1.72**	**0.56**	**0.71**	**0.48**
TRT 4 (Applications at 6-leaf, first white flower + 15 days, first open boll, and 7 days prior to harvest; EP: Clarity)											
Gin byproducts	Dicamba	2.0 (2.2)	6-7	3	3.09	23.6	3.13	23.0	14.9	13.7	10.0
	5-OH Dicamba			3	<0.04	0.04	<0.04	<0.04	0.04	0.04	N/A
	DCSA			3	1.70	6.29	1.78	6.17	4.50	4.15	2.22
	Combined Residues			**3**	**<4.83**	**29.9**	**<5.06**	**<29.6**	**19.7**	**18.1**	**12.2**
TRT 5 (Applications at 6-leaf, first white flower + 15 days, first open boll, and 7 days prior to harvest; EP: MON 11968)											
Undelinted Cotton seed	Dicamba	2.0 (2.2)	7-8	4	0.17	0.72	0.20	0.62	0.41	0.41	0.23
	5-OH Dicamba			4	<0.02	<0.02	<0.02	<0.02	0.02	0.02	N/A
	DCSA			4	0.02	0.17	0.02	0.12	0.04	0.06	0.04
	Combined Residues			**4**	**<0.21**	**<0.91**	**<0.24**	**<0.76**	**0.47**	**0.49**	**0.27**

[1] Except for sample min/max, values reflect per trial averages; n = no. of field trials. For calculation of median, mean, and standard deviation, the LOQ (0.02 ppm each analyte in undelinted cotton seed and 0.04 ppm for each analyte in cotton gin byproducts) was used for any results reported as <LOQ in Table C.3. Combined residues of dicamba, 5-OH dicamba, DCGA, and DCSA are expressed in parent equivalents. Individual analyte results are reported as per se. N/A = Not applicable.

[2] LAFT = lowest-average-field-trial; HAFT = highest-average-field-trial.

Conclusions. The submitted crop field trial results provided by Monsanto for MON 88701 cotton treated with dicamba are adequate to fulfill data requirements. The number and location of these field trials are sufficient in meeting the OCSPP 860.1500 Residue Chemistry Test Guidelines for determining the magnitude of the residue in/on cotton RACs. There were no unusual meteorological conditions during the course of the field trials which would adversely affect the quality and integrity of the study. An acceptable method was used for quantitation of residues in/on cotton RACs and adequate supporting storage stability data are available.

860.1520 Processed Food and Feed

DER Reference: 48728703.der2.docx (Magnitude of Dicamba Residues in Dicamba-Tolerant Cotton)

Monsanto has submitted processing study data for dicamba-tolerant MON 88701 cotton treated with dicamba. Samples used for processing were generated from the two (2) trials held in Missouri and Texas during the 2010 growing season discussed above. Each trial site maintained two treated test plots which received the maximum total seasonal application rate of 2.0-2.1 lb ae/A applied following one (1) of two (2) distinct use patterns evaluated for study.

All applications were made at the maximum labeled rate with the 4 lb ae/gal SL diglycolamine salt formulation of dicamba. Treatments were made using ground equipment at spray volumes of 19.7-20.5 gal/A (184-192 l/ha) and included a nonionic surfactant (NIS) and ammonium sulfate (AMS) adjuvants. Samples of cotton undelinted seed were harvested using a mechanical picker at maturity which followed a PHI of 71-79 days for TRT 2 and 6-7 days for TRT 4. The resulting cotton undelinted seed samples were then processed into meal, hulls, alkali-refined oil, and RBD (Refined Bleached Deodorized) oil by GLP Technologies located in Navasota Texas using procedures simulating commercial processing.

To analyze for residues of dicamba, the cotton RAC and its processed commodities were determined using the Monsanto LC/MS/MS method AG-ME-1381-01. The reported LOQ for this LC/MS/MS method is 0.02 ppm for each analyte in cotton undelinted seed as well as for its processed commodities. The method was successfully validated prior to and in conjunction with the analysis of the field trial samples. As previously noted, this analytical method does not specify protocol for conversion of metabolite residues to parent equivalents. The quantifiable residues of 5-OH dicamba, DCSA, and DCGA were converted to parent equivalents using molecular weight conversion factors of 0.933, 1.068, and 0.991, respectively.

For the two use patterns tested in this study, quantifiable residues were only obtained for the four post-emergence applications comprising TRT 4. Using these data, the concentration factors for each dicamba residue of concern was calculated for the processed commodities of dicamba-tolerant cotton seed. These data indicate that the combined residues of dicamba do not concentrate in hulls, meal, alkali refined oil, or RBD oil by yielding processing factors of $\leq$1.0x for each dicamba analyte, respectively. A summary of the data acquired from these processing studies is presented below in Table 8.

Table 8. Summary of Residue Data from the Cotton Processing Study made with Dicamba.									
RAC: MRID No.	Processed Commodity	Total Rate lb ae/A (Kg ae/A)	TRT[1]	Residues[2] (ppm)			Avg. Processing Factor[3]		
				Parent	5-OH	DCSA	Parent	5-OH	DCSA
Cotton/ MRID No. 48728703	Undelinted Seed (RAC)	2.0 (2.2)	4	0.65	<0.02	0.04	--	--	--
	Hulls			0.43	<0.02	0.04	0.66x	NC	1.0x
	Meal			<0.02	<0.02	<0.02	<0.03x	NC	<0.5x
	Alkali Refined Oil			<0.02	<0.02	<0.02	<0.03x	NC	<0.5x
	RBD Oil			<0.02	<0.02	<0.02	<0.03x	NC	<0.5x

[1] TRT – notes the use pattern that was followed for study.
[2] Residues of dicamba include parent along with the 5-OH dicamba and DCSA metabolites of concern expressed in parent equivalents obtained for the TRT 4 use pattern trials. The LOQ is 0.02 ppm for each analyte in every given matrix. Concentrations found at or below the LOQ are denoted as < 0.02 ppm and included in the totals as 0.02 ppm.
[3] NC – "not calculated" since there are not quantifiable residues in the RAC.

Conclusions: The cotton processing study is adequate to satisfy OCSPP 860.1520 data requirements. Residues of dicamba do not concentrate in the processed commodities of dicamba-tolerant (MON 88701) cotton. Therefore, separate tolerances are not required for the processed commodities of dicamba-tolerant (MON 88701) cotton.

860.1650 Submittal of Analytical Reference Standards

Analytical standards for dicamba and its metabolites of concern are currently available in the EPA National Pesticide Standards Repository (personal communication with Theresa Cole, BEAD, 01/30/2013). The current stock of standards is set to expire on 11/30/2015.

860.1850/860.1900 Confined and Field Accumulation in Rotational Crops

Reregistration Eligibility Decision Memo, D317699, 12/20/2005, C. L. Olinger

The December 2005 Reregistration Eligibility Decision (RED) for dicamba requires additional rotational crop studies in order to satisfy data requirements. However, these data are not needed if a 120-day plantback interval is specified when dicamba is applied at the maximum seasonal rate of 0.75 lb ae/A. For greater seasonal application rates of 0.75-2.0 lb ae/A, only crops with established tolerances can be rotated for planting. Because no new rotational crop data have been submitted, the plantback restrictions noted in the 2005 RED are required, and have been appropriately reflected on the proposed product label for dicamba-tolerant (MON 88701) cotton.

860.1550 Proposed Tolerances

Permanent tolerances are established under 40 CFR §180.227(a)(1) for dicamba and its 3,6-dichloro-5-hydroxybenzoic acid (5-OH dicamba) metabolite. There are also additional tolerances listed under 40 CFR §180.227(a)(2) for dicamba and its 3,6-dichloro-2-hydroxybezoic acid metabolite, and under 40 CFR §180.227(a)(3) for dicamba, and the 5-OH dicamba and DSCA metabolites. The tolerance expression recommended for cotton commodities based on the new metabolism data provided in support of this action is parent, 5-OH dicamba, and DSCA established under 40 CFR §180.227(a)(3). However, the 40 CFR §180.227(a)(1) citation for dicamba currently lists a 0.2 ppm tolerance for cotton, undelinted seed. This registration action supersedes the 40 CFR §180.227(a)(1) cotton undelinted seed tolerance which requires it to be removed from the federal register. Therefore all

tolerances for cotton RACs are now to be established under 40 CFR §180.227(a)(3) as recommended above.

The International Residue Limit (IRL) summary is shown in Attachment 1 of this memorandum. There are MRLs of 0.2 ppm in Mexico and 0.04 ppm established by Codex on cotton seed currently established. Mexico adopts existing U.S. crop tolerances for export purposes which in this instance is the current cotton undelinted seed tolerance of 0.2 ppm. There are currently no Mexican, Canadian or Codex MRLs established for cotton gin byproducts. Because the registrant is now requesting a late season use of dicamba on dicamba-tolerant cotton, the currently established international tolerances are not adequate to cover residues likely from the newly proposed use in the U.S. In addition, the dicamba residues of concern for dicamba-tolerant cotton also include the DCSA metabolite which is not found nor regulated in the other common varieties of cotton. Therefore, harmonization with respect to the tolerance expression is not possible at this time for cotton seed. Since there are no international tolerances on cotton gin byproducts, there is no issue of international harmonization relevant to that tolerance.

The cotton field trial residue data reflecting late season treatments at the labeled 7-day PHI minimum (TRT 3 & TRT 4) were evaluated by the OECD calculation procedures to determine an appropriate tolerance level (see Appendix III) to yield a recommended tolerance of 3.0 ppm for cotton undelinted seed. Using the OECD calculation procedures, the cotton gin by products data reflecting late season treatments at the labeled 7-day PHI minimum (TRT 4) were also evaluated and a tolerance of 70.0 ppm is recommended (see Appendix III). As previously noted , no concentration in cotton seed processed commodities was seen; therefore, separate tolerances are not required for cotton processed commodities. The final tolerance recommendations are shown below in Table 9.

Table 9. Tolerance Summary for Dicamba.

Commodity	Established Tolerance (ppm)	Proposed Tolerance (ppm)	Recommended Tolerance (ppm)	Comments; Correct Commodity Definition
Tolerances Proposed for 40 CFR §180.227(a)(3)				
Cotton, gin byproducts	--	70.0	70.0	
Cotton, undelinted seed	0.2	3.0	3.0	

References

DP No.: D317699
Subject: Dicamba. Residue Chemistry Considerations for the Reregistration Eligibility Decision (RED) Document. Summary of Analytical Chemistry and Residue Data.
From: C. L. Olinger
To: K. Tyler
Date: 12/20/2005
MRIDs: None

DP No.: None
Subject: Dicamba Registration Standard
From: C. L. Trichilo
To: A. Rispin and R. Taylor
Date: 08/12/1983
MRIDs: None

DP No.: D320564 and D322842
Subject: Reregistration of Dicamba. Livestock Storage Stability Study and Ruminant Feeding Study
From: C. L. Olinger
To: K. Tyler
Date: 12/08/2005
MRIDs: 44891303 and 46668101

Attachments:
International Residue Limit Status Sheet
Appendix I - Characterization and Identification of Radioactive Residues in Dicamba-Tolerant Cotton
Appendix II - Chemical Names and Structures of Dicamba and its Metabolites
Appendix III - Tolerance Assessment Calculations

International Residue Limits

Dicamba (029801, 029802, 029806, 128931, 128944 & 129043; 01/16/2013)

Summary of US and International Tolerances and Maximum Residue Limits			
Residue Definition[1]:			
US	Canada	Mexico[2]	Codex[3]
40 CFR 180.227: Plant: Compliance with the tolerance levels is to be determined by measuring only the residues of dicamba, 3,6-dichloro-0-anisic acid, and its metabolites, 3,6-dichloro-5-hydroxy-0-anisic acid, and 3,6-dichloro-2-hydroxybenzoic acid, calculated as the stoichiometric equivalent of dicamba	benzoic acid, 3,6-dichloro-2-methoxy-, including the metabolite benzoic acid, 2,5-dichloro-3-hydroxy-6-methoxy-		Plants: Dicamba Animals: sum of dicamba and 3,6-dichlorosalicylic acid (DCSA) expressed as dicamba. The residue is not fat-soluble.

Commodity[4]	*Tolerance (ppm) /Maximum Residue Limit (mg/kg)*			
	US	Canada	Mexico[2]	Codex[3]
Cotton, undelinted seed	3			0.04 cotton seed (*)
Cotton, gin byproducts	70			
Completed: M. Negussie; 01/17/2013				

[1] The U.S. Residue definition for cotton commodities now includes the DCSA metabolite not found nor regulated in the common varieties of cotton.

[2] Mexico adopts US tolerances and/or Codex MRLs for its export purposes.

[3] * = absent at the limit of quantitation; Po = postharvest treatment, such as treatment of stored grains. PoP = processed postharvest treated commodity, such as processing of treated stored wheat. (fat) = to be measured on the fat portion of the sample. MRLs indicated as proposed have not been finalized by the CCPR and the CAC.

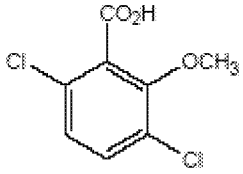
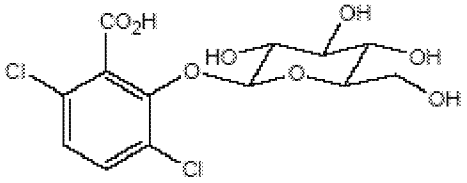
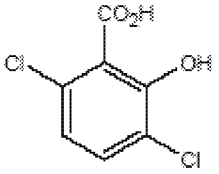
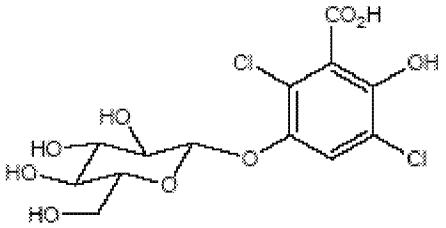
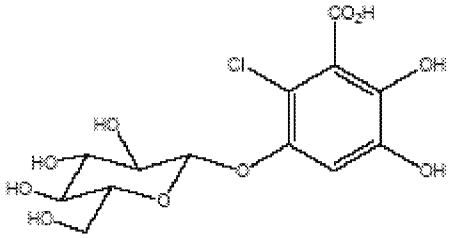
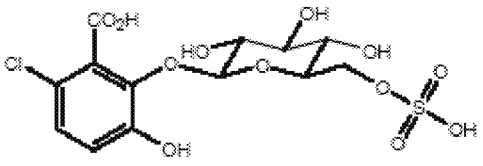
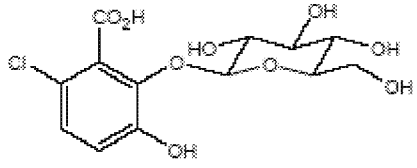
[4] Includes only commodities of interest for this action. Tolerance values should be the HED recommendations and not those proposed by the applicant.

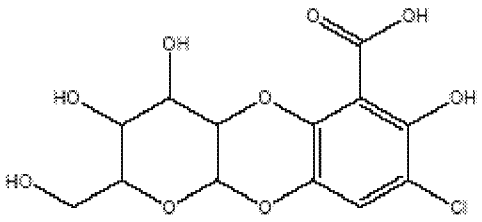
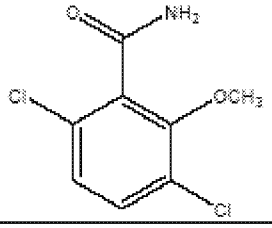
Appendix I – Characterization and Identification of Radioactive Residues in Dicamba-Tolerant Cotton

Appendix I. Summary of Characterization and Identification of Radioactive Residues in Dicamba-Tolerant Cotton Matrices Following Application of [Phenyl-U-^{14}C]dicamba at 2.094 lb ae/A (preemergence) or 2.065 lb ae/A (postemergence)

	Preemergence				Postemergence			
	Seed		Gin byproducts		Seed		Gin byproducts	
	TRR = 0.1621 ppm		TRR = 0.8493 ppm		TRR = 0.9778 ppm		TRR = 60.024 ppm	
	% TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Dicamba	0.09	0.0001	0.50	0.004	0.85	0.008	4.48	2.691
DCSA glucoside	0.73	0.0012	27.77	0.236	3.42	0.033	16.83	10.101
DCSA	0.06	0.0001	5.42	0.046	1.91	0.019	13.39	8.035
DCGA glucoside	0.09	0.0001	0.68	0.006	1.1	0.011	2.46	1.476
MCTHBA glucoside	--	--	--	--	--	--	3.33	1.998
MCDHBA glucoside sulfate	--	--	--	--	--	--	4.71	2.828
MCDHBA glucoside	--	--	--	--	--	--	0.84	0.505
MCTHBA cyc. glucoside	--	--	2.94	0.025	--	--	2.76	1.656
Dicamba amide	--	--	3.22	0.027	--	--	--	--
Fatty acids	16.05	0.026	--	--	10.07	0.098	--	--
Glycerol	2.86	0.005	--	--	1.35	0.013	--	--
Sugars/natural products	4.59	0.0074	8.94	0.076	5.61	0.055	2.69	1.613
Phosphate rinse	3.87	0.006	3.25	0.028	1.96	0.019	6.20	3.72
Starch	2.79	0.005	2.73	0.023	2.19	0.021	7.05	4.234
Protein	17.71	0.029	1.71	0.015	10.10	0.099	2.25	1.350
Pectin	7.40	0.012	1.91	0.016	4.04	0.039	2.66	1.595
Lignin	6.18	0.010	5.28	0.045	14.00	0.136	6.60	3.962
Cellulose	4.37	0.007	0.90	0.008	3.13	0.031	0.62	0.374
Hemicellulose	14.43	0.023	5.35	0.045	13.49	0.132	2.80	1.679
Acid Hydrolysates	5.07	0.008	--	--	3.21	0.031	--	--
Unidentified (oil)	1.94	0.003	--	--	0.84	0.008	--	--
Discrete unknowns	3.03	0.0049	15.96	0.136	5.36	0.052	0.86	0.518
Total identified	0.97	0.0015	40.53	0.344	7.28	0.071	48.80	29.29
Total characterized	90.29	0.1463	46.03	0.392	75.35	0.734	31.73	19.045
Total extractable	92.65	0.150	97.38	0.827	90.34	0.883	99.39	59.659
Unextractable (PES)[1]	7.35	0.012	2.62	0.022	9.66	0.094	0.61	0.365
Accountability[2]	100		100		100		100	

Appendix II - Chemical Names and Structures of Dicamba and its Metabolites

Appendix II. Summary of Chemical Names and Structures of Dicamba and its Metabolites.		
Common name/code	Chemical name	Chemical structure
Dicamba	3,6-dichloro-2-methoxybenzoic acid	
DCSA Glucoside	3,6-dichloro-2-(β-D-glucopyranosyloxy)benzoic acid	
DCSA	3,6-dichloro-2-hydroxy benzoic acid	
DCGA Glucoside	2,5-dichloro-3-(β-D-glucopyranosyloxy)-6-hydroxybenzoic acid	
MCTHBA Glucoside	2-Chloro-5,6-dihydroxy-3-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydropyran-2-yloxy)benzoic acid	
MCDHBA Glucoside Sulfate	6-Chloro-3-hydroxy-2-(3,4,5-trihydroxy-6-sulfooxymethyltetrahydro-pyran-2-yloxy)benzoic acid	
MCDHBA Glucoside	6-Chloro-3-hydroxy-2-(3,4,5-trihydroxy-6-hydroxymethyltetrahydro-pyran-2-yloxy)benzoic acid	

Appendix II. Summary of Chemical Names and Structures of Dicamba and its Metabolites.		
Common name/code	Chemical name	Chemical structure
MCTHBA Cyclic Glucoside	7-Chloro-3,4,6-trihydroxy-2-hydroxymethyl-3,4,4a,9a-tetrahydro-2*H*-1,9.10-trioxa-anthracene-5-carboxylic acid	
Dicamba Amide	3,6-dichloro-2-methoxybenzamide	

Appendix III. Tolerance Assessment Calculations.

Cotton, undelinted seed

The dataset used to establish a tolerance for dicamba on cotton undelinted seed consisted of field trial results held in the representative NAFTA Growing Zones during the 2010 growing season. It was acquired to evaluate the registration of dicamba for post-emergence use on dicamba-tolerant cotton since this variety can tolerate application of the herbicide in this manner. The data reflect the maximum seasonal application rate of 2.0 lb ae/A or 64 fluid ounces per acre in a given year following a 7-day RTI and a 7-day PHI. Treatments were made with a 4.0 lb ae/gal SL diglycolamine salt product which included a non-ionic surfactant and ammonium sulfate added to the spray mixture. These field trial data quantified the expected range of residues in cotton undelinted seed following treatment according to the proposed use pattern. All cotton undelinted seed samples had combined residues of dicamba above the LOQ of 0.02 ppm.

To establish an appropriate tolerance level on this cotton RAC, only the data acquired using late season treatments at the labeled 7-day PHI minimum (TRT 3 & TRT 4) were entered into the Organization for Economic Cooperation and Development (OECD) calculation procedures for determination. For those field trials where sampling replicates were taken, the average or mean value was used for calculation. The mean residue values used to calculate the resulting tolerance level is provided in the table below. When using the OECD calculation procedures, the resulting MRL value is always found to be greater than or equal to the highest residue within the dataset. MRLs are displayed without decimal zeros after the last significant figure and are rounded in the last step of calculation to produce a result in the region of the 95th percentile. The MRL estimate calculated at the "CF x 3 Mean" level gives a value of 3.0 ppm for the combined residues of dicamba.

Dicamba
Cotton, undilented seed
U.S.
PHI= 6-8 days

Total number of data (n)	26
Percentage of censored data	0%
Number of non-censored data	26
Lowest residue	0.110
Highest residue	1.720
Median residue	0.700
Mean	0.726
Standard deviation (SD)	0.458
Correction factor for censoring (CF)	1.000
Proposed MRL estimate	
- Highest residue	1.720
- Mean + 4 SD	2.560
- CF x 3 Mean	2.178
Unrounded MRL	2.560
Rounded MRL	**3**

Residues (mg/kg)	n
0.11	1
0.16	1
0.17	1
0.19	1
0.23	1
0.26	1
0.36	1
0.38	1
0.43	1
0.46	1
0.52	2
0.68	1
0.72	1
0.75	1
0.8	1
0.92	1

1	1
1.05	1
1.07	1
1.09	1
1.11	1
1.26	1
1.35	1
1.57	1
1.72	1

Cotton, gin byproducts

The dataset used to establish a tolerance for dicamba on cotton gin byproducts consisted of field trial results held in the representative NAFTA Growing Zones during the 2010 growing season. It was acquired to evaluate the registration of dicamba for post-emergence use on dicamba-tolerant cotton since this variety can tolerate application of the herbicide in this manner. The data reflect the maximum seasonal application rate of 2.0 lb ae/A or 64 fluid ounces per acre in a given year following a 7-day RTI and a 7-day PHI. Treatments were made with a 4.0 lb ae/gal SL diglycolamine salt product which included a non-ionic surfactant and ammonium sulfate added to the spray mixture. These field trial data quantified the expected range of residues in cotton gin byproducts following treatment according to the proposed use pattern. All cotton gin byproducts samples had combined residues of dicamba above the LOQ of 0.04 ppm.

To establish an appropriate tolerance level on this cotton RAC, only the data acquired using late season treatments at the labeled 7-day PHI minimum (TRT 4) were entered into the Organization for Economic Cooperation and Development (OECD) calculation procedures for determination. For those field trials where sampling replicates were taken, the average or mean value was used for calculation. The mean residue values used to calculate the resulting tolerance level is provided in the table below. When using the OECD calculation procedures, the resulting MRL value is always found to be greater than or equal to the highest residue within the dataset. MRLs are displayed without decimal zeros after the last significant figure and are rounded in the last step of calculation to produce a result in the region of the 95th percentile. The MRL estimate calculated at the "CF x 3 Mean" level gives a value of 70.0 ppm for the combined residues of dicamba.

Dicamba
Cotton, gin byproducts
U.S.
PHI= 6-8 days

Total number of data (n)	3
Percentage of censored data	0%
Number of non-censored data	3
Lowest residue	5.060
Highest residue	29.600
Median residue	19.700
Mean	18.120
Standard deviation (SD)	12.346
Correction factor for censoring (CF)	1.000

Proposed MRL estimate

- Highest residue	29.600
- Mean + 4 SD	67.504
- CF x 3 Mean	54.360
Unrounded MRL	67.504
Rounded MRL	**70**

High uncertainty of MRL estimate.
[Small dataset]

Residues (mg/kg)	n
5.06	1
19.7	1
29.6	1

Primary Evaluator		Date: 11/15/2012
	Versar, Inc.	
Approved by	Peter Savoia	Date: 04/22/2013
	Peter Savoia, Chemist, OCSPP-HED-RAB V/VII	

Note: This Data Evaluation Record (DER) was originally prepared under contract by Versar, Inc. (6850 Versar Center, Springfield, VA 22151; submitted 11/15/2012). The DER has been reviewed by HED and revised to reflect current Office of Pesticide Programs (OPP) policies.

STUDY REPORT:

48728701 Whitehead, T.; Mierkowski, M.; Chott, R. (2011) Amended Report for MSL0021858: Metabolism of (Carbon 14)-Dicamba in Dicamba-Tolerant Cotton. Project Number: MSL0023760/OCR, REG/09/046, 1901W. Unpublished study prepared by Monsanto Company. 378 p.

EXECUTIVE SUMMARY:

Monsanto has submitted a study investigating the metabolism of uniformly ring-labeled [phenyl-U-^{14}C]dicamba (specific activity of 9.85-9.91 mCi/mmol) in dicamba- and glufosinate-tolerant cotton, identified as Event MON 88701 (formerly GH_S26695). Event MON 88701 cotton expresses a modified dicamba mono-oxygenase (DMO) gene derived from *Stenotrophomonas maltophilia* and the bialaphos resistance (BAR) gene isolated from *Streptomyces hygroscopicus*. The expression of DMO confers dicamba tolerance, and the expression of BAR confers glufosinate tolerance. The radiolabeled test substance was formulated as an aqueous solution of the diglycolamine (DGA) salt and applied to cotton grown in outdoor box plots. One plot received a single pre-emergence soil application (PRE-T) at 2.094 lb ae/A on the day of planting after seed was planted, and the other plot received a single post-emergence foliar application (POE-T) at 2.065 lb ae/A at the first white flower stage (76 days after planting). Samples of seed cotton and leaves/stems (surrogate gin byproducts) were collected at maturity: 180 days after treatment (DAT) for the PRE-T plot and 104 DAT for the POE-T plot. The in-life phase of the study was conducted by PTRL West, Inc. (Hercules, CA) and Exeel Research Services (Fresno, CA), and the analytical phase of the study was conducted by Monsanto (St. Louis, MO).

Total radioactive residues (TRR) were determined by combustion/LSC. Following a single pre-emergence soil application of [^{14}C]dicamba at 2.094 lb ae/A, TRR were 0.1621 ppm in seed and 0.8493 ppm in gin byproducts harvested at a 180-day pre-harvest interval (PHI). Following a single post-emergence foliar application at 2.065 lb ae/A, TRR were 0.9778 ppm in seed and 60.0235 ppm in gin byproducts harvested at a 104-day PHI.

Extraction with hexane released 18.91% and 11.47% TRR from PRE-T and POE-T seed, respectively; extraction with acetonitrile (ACN) and ACN/water together released an additional 11.91% and 26.78% TRR, respectively. In gin byproducts, extraction with ACN/water released the majority of the radioactivity: 76.25% and 71.21% TRR from PRE-T and POE-T gin byproducts, respectively. Remaining non-extractable residues for all matrices were subjected to

sequential enzymatic and chemical hydrolysis and oxidation procedures, including a phosphate rinse followed by hydrolysis with α-amylase and protease; EDTA extraction; oxidation with chlorite; hydrolysis with cellulase, 24% aqueous KOH, and sulfuric acid (seed only); a second hydrolysis with protease (POE-T seed only); and hot DMSO extraction (POE-T seed only). These procedures released 52.12-61.82% TRR in seed and 21.13-28.18% TRR in gin byproducts. Non-extractable residues remaining following hydrolysis procedures were 7.35% TRR (0.012 ppm) in PRE-T seed, 9.66% TRR (0.094 ppm) in POE-T seed, 2.62% TRR (0.022 ppm) in PRE-T gin byproducts, and 0.61% TRR (0.365 ppm) in POE-T gin byproducts. These procedures adequately extracted the majority of residues (90.3-99.4%) from all cotton matrices. Extraction results were normalized; therefore, accountabilities were ~100%.

Up to 38 peaks were observed in the initial metabolic profiles of seed and gin byproducts. All metabolite peaks accounting for ≥2% TRR were isolated and purified by preparative high performance liquid chromatography (HPLC) for further investigation. After isolation, residues were identified by HPLC/UV and by mass spectral analysis including LC/MS and LC/MS/MS; Gas chromatography/electron ionization mass spectrometry (GC/EI/MS) was used for volatile derivatives of the metabolites or their hydrolysis products. Isolated metabolites were subjected to partitioning with ethyl acetate, acid, base or enzymatic hydrolysis, and/or chemical derivatizations (methylation and acetylation) to provide additional structural information. Comparison of the chromatographic and mass spectral properties of the metabolite derivatives or hydrolysates to those of synthetic reference standards was used to confirm the identities of the metabolites. The study is supported by adequate storage stability data.

Parent dicamba was identified in all matrices at low levels: 0.09-0.85% TRR (0.0001-0.008 ppm) in seed and 0.50-4.48% TRR (0.004-2.691 ppm) in gin byproducts. Metabolites DCSA glucoside, DCSA, and DCGA glucoside were also present in all matrices. DCSA glucoside was the major identified metabolite in gin byproducts, at 16.83-27.77% TRR (0.236-10.101 ppm) and was present in seed at low levels (0.73-3.42% TRR). Free DCSA was the next most abundant metabolite in POE-T gin byproducts (13.39% TRR, 8.035 ppm) and was present in PRE-T gin byproducts at 5.42% TRR and in seed at 0.06-1.91%TRR. DCGA glucoside was identified at low levels in all matrices (0.09-2.46% TRR). The remaining identified metabolites were found in gin byproducts only and accounted for ≤4.71% TRR each, including MCTHBA glucoside, MCDHBA glucoside sulfate, and MCDHBA glucoside in POE-T gin byproducts, MCTHBA cyclic glucoside in both PRE-T and POE-T gin byproducts, and dicamba amide in PRE-T gin byproducts only. Because dicamba amide was only observed in PRE-T plants, it is believed to be a soil metabolite that was taken up by the plants.

Remaining extractable radioactivity in cotton seed and gin byproducts was characterized as triglycerides and sugars/natural products. In the hexane extracts of seed, triglycerides including fatty acids (palmitic, stearic, linoleic, and oleic) accounted for 10.07-16.05% TRR (0.026-0.098 ppm), while glycerol accounted for 1.35-2.86% TRR. In the ACN/water extracts of all matrices, sugars/natural products (Peak 1) accounted for 2.69-8.94% TRR (0.0074-1.613 ppm). This peak was characterized extensively, indicating that it was a mixture of polar materials, including sugars such as glucose, sucrose, and raffinose, and amino acids such as phenylalanine and glutamic acid. Unidentified components in oil accounted for 0.84-1.94% TRR in seed; discrete

unknowns in the ACN/water extracts together accounted for 0.86-15.96% TRR (0.0049-0.518 ppm) in seed and gin byproducts, with none present at >2.91% TRR.

Radioactivity in non-extractable residues was characterized by sequential enzymatic and chemical hydrolysis/oxidation. In seed, these procedures indicated that the majority of radioactivity was incorporated into hemicellulose (13.49-14.43% TRR, 0.023-0.132 ppm), protein (10.10-17.71% TRR, 0.029-0.099 ppm), and lignin (6.18-14.0% TRR, 0.01-0.136 ppm). Other fractions (including starch, pectin, and cellulose) individually accounted for ≤7.4% TRR. In gin byproducts, radioactivity was incorporated primarily into starch (2.73-7.05% TRR, 0.023-4.234 ppm), lignin (5.28-6.60% TRR, 0.045-3.962 ppm), and hemicellulose (2.80-5.35% TRR, 0.045-1.679 ppm). Other fractions (including protein, pectin, and cellulose) individually accounted for ≤6.2% TRR.

Based on the results of the metabolism study on dicamba-tolerant cotton, the petitioner proposes that the metabolism of dicamba proceeds by initial demethylation of dicamba to form DCSA through the action of the dicamba *O*-demethylase enzyme, which is the product of the DMO gene introduced to confer dicamba tolerance. The petitioner noted that, due to the predominance of the *O*-demethylation pathway, 5-OH-dicamba is not observed as a metabolite in dicamba-tolerant cotton or soybean. While free DCSA was observed in cotton matrices (POE-T and PRE-T gin byproducts in particular), the majority of DCSA is found as its conjugate DCSA glucoside. Some DCSA glucoside is further modified by hydroxyl replacement of chlorine to form MCDHBA glucoside which is then further conjugated by sulfation to form MCDHBA glucoside sulfate. In a minor pathway, DCSA is hydroxylated at the 5-position, presumably by a P-450 enzyme or other oxygenase, to form DCGA. DCGA is not observed as a free metabolite in cotton matrices but is converted to DCGA glucoside in which the glucose moiety is attached to the hydroxyl group at the 5-position of DCGA (based upon comparison to the same metabolite identified in dicamba-tolerant soybean). DCGA glucoside is further converted to MCTHBA glucoside by hydroxyl replacement of chlorine at the 3-position. Alternatively, MCTHBA cyclic glucoside may form from DCGA glucoside through displacement of chlorine at the 6-position of the DCGA portion of the molecule by a hydroxyl group of the glucose moiety. Dicamba amide was a minor metabolite that was only observed in PRE-T matrices. The petitioner concluded that this metabolite is most likely the result of the metabolic conversion of the carboxylic acid moiety of dicamba to an amide functional moiety *via* soil microbial metabolism.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the plant metabolism data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document D408384.

COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Dicamba (3,6-dichloro-2-methoxybenzoic acid) is a selective benzoic acid herbicide registered for the control of weeds prior to their emergence. The dicamba Reregistration Eligibility Decision (RED) was issued December 2005. The chemical structure and nomenclature of dicamba and its metabolites 5-OH dicamba, DSCA, and DCGA are presented in Table A.1. The physicochemical properties of the technical grade of dicamba acid are presented in Table A.2.

TABLE A.1. Test Compound Nomenclature.

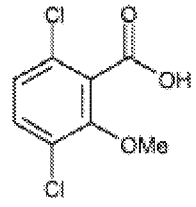
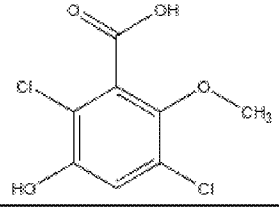
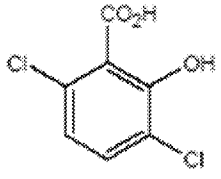
Compound	
Common name	Dicamba
Company experimental name	MON 11900
IUPAC name	3,6-dichloro-o-anisic acid or 3,6-dichloro-2-methoxybenzoic acid
CAS name	3,6-dichloro-2-methoxybenzoic acid
CAS registry number	1918-00-9 (dicamba acid), 104040-79-1 (diglycolamine salt), or 53404-28-7 (monoethanolamine salt)
End-use product	Not applicable.
Compound	
Common name	5-Hydroxy-dicamba
Company experimental name	5-OH dicamba
IUPAC/CAS name	2,5-dichloro-3-hydroxy-6-methoxybenzoic acid
CAS registry number	7600-50-2
Compound	
Common name	DCSA; 3,6-dichlorosalicylic acid
Company experimental name	MON 52708
IUPAC/CAS name	3,6-dichloro-2-hydroxybenzoic acid
CAS registry number	3401-80-7

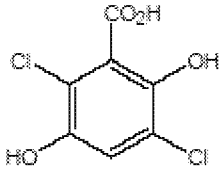
TABLE A.1. Test Compound Nomenclature.	
Compound	
Common name	DCGA; 3,6-dichlorogentistic acid
Company experimental name	MON 52724
IUPAC/CAS name	2,5-dichloro-3,6-dihydroxybenzoic acid
CAS registry number	18688-01-2

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound: Dicamba.		
Parameter	Value	Reference
Melting point	114-116 EC (PAI) 90-100 EC (87% TGAI)	Residue Chemistry Chapter of the Dicamba RED, DP# 317699, 12/20/05, C. Olinger
pH	2.5-3.0 (87% TGAI)	
Density	1.57 g/mL at 25 EC (87% TGAI)	
Water solubility	0.5 g/100 mL at 25 EC (PAI)	
Solvent solubility	g/100 mL at 25 EC (PAI) dioxane 118.0 ethanol 92.2 isopropyl alcohol 76.0 methylene chloride 26.0 acetone 17.0 toluene 13.0 xylene 7.8 heavy aromatic naphthalene 5.2	
Vapor pressure	3.4 x 10^{-5} mm Hg at 25 EC (PAI)	
Dissociation constant, pK_a	1.97 (PAI)	
Octanol/water partition coefficient, Log(K_{OW})	0.1 (PAI)	
UV/visible absorption spectrum	neutral: 511 (275 nm) acidic (pH 0-1): 1053 (281 nm) basic (pH 13-14): 469 (274 nm)	

B. EXPERIMENTAL DESIGN

B.1. Test Site and Crop Information

The in-life and analytical phases of the study were conducted by PTRL West, Inc. (Hercules, CA) and Excel Research Services (Fresno, CA). Cotton was grown from seed planted in loamy sand soil in outdoor box plots. Separate boxes were used for pre- and post-emergence applications. The plots were located in Madera, CA.

Crops were maintained under normal agricultural conditions. Fertilizer was applied during the study period; however, maintenance pesticides were not applied. Weather data for the site was provided, including minimum/maximum temperatures and daily rainfall. Weather conditions were reported to be normal for the study duration. When rain threatened, the plots were covered

so that no rain fell on the plots. Plots were watered by hand as needed. Soil characteristics are presented in Table B.1.1, and crop information is presented in Table B.1.2.

TABLE B.1.1. Test Site Information.

Type	Method	Soil characteristics			
		Type	%OM	pH	CEC meq/100g
Preemergence soil treatment (PRE-T)	Cotton was grown from seed in a 4 ft. x 4 ft. (16 ft^2; PRE-T) or 3 ft. x 3 ft. (9 ft^2; POE-T) wooden box filled with ~18 in of soil and lined with heavy- gauge plastic.	Loamy sand	0.2	6.8	6.3
Postemergence foliar treatment (POE-T)					

TABLE B.1.2. Crop Information.

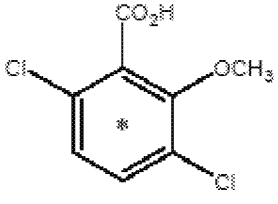
Crop/crop group	Variety[1]	Growth stage at application	Growth stage at harvest	Harvested Matrix
Cotton; Oilseed group 20	Event MON 88701	PRE-T: day of planting after planting seed	Maturity	Seed; leaves and stems (gin byproducts)
		POE-T: first white flower stage (50% of the plants had first white flower)		

[1] Dicamba- and glufosinate-tolerant cotton was used in the study, identified as Event MON 88701 (formerly GH_S26695). Event MON 88701 cotton expresses a modified dicamba mono-oxygenase (DMO) gene derived from *Stenotrophomonas maltophilia* and the bialaphos resistance (BAR) gene isolated from *Streptomyces hygroscopicus*. The expression of DMO confers dicamba tolerance, and the expression of BAR confers glufosinate tolerance to MON 88701

B.2. Test Materials

The radiolabeled test substance, [phenyl-U-^{14}C]dicamba (specific activity 45.0 mCi/mmol, 7.53 mBq/mg), was dissolved in ACN and isotopically diluted with unlabeled dicamba. The characteristics of the test substance are presented in Table B.2.1.

TABLE B.2.1. Test Material Characteristics.

	Preemergence Treatment	Postemergence Treatment
Chemical structure		
Radiolabel position	[Phenyl-U-^{14}C]dicamba	
Lot No.	2344-2	2344-9
Purity	98.4% prior to and after application	98.0% prior to application; 98.1% after application
Specific activity	9.91 mCi/mmol (99548 dpm/µg or 1.66 MBq/mg)	9.85 mCi/mmol (98946 dpm/µg, 1.65 MBq/mg)

B.3. Study Use Pattern

The isotopically diluted test substance was formulated as an aqueous solution of the DGA salt and diluted with water. Applications were made using a hand-held sprayer. Applications were made as a single pre-emergence soil broadcast application (PRE-T) at 2.094 lb ae/A on the day

of planting after seed was planted or as a single post-emergence foliar application (POE-T) at 2.065 lb ae/A 76 days after planting, when >50% of the plants had reached the first white flower growth stage. Samples of seed and leaves/stems were harvested at PHIs of 180 days for the PRE-T plot and 104 days for the POE-T plot. The study use pattern is summarized in Table B.3.1.

TABLE B.3.1. Use Pattern Information.

Chemical name	[Phenyl-U-^{14}C]dicamba
Application method	The test material was formulated as an aqueous solution of the DGA salt, diluted with water, and applied as a single preemergence soil application (PRE-T plot) or a single postemergence foliar application (POE-T plot).
Application rate	PRE-T: 2.094 lb ae/A (2,347 g ae/ha) POE-T: 2.065 lb ae/A (2,315 g ae/ha)
Number of applications	PRE-T: 1 POE-T: 1
Timing of application	PRE-T: Single preemergence soil application made on the day of planting after seed was planted POE-T: Single postemergence application made at the first white flower stage (76 days after planting).
PHI	PRE-T: 180 days POE-T: 104 days

B.4. Identification/ Characterization of Residues

B.4.1. Sample Handling and Preparation

Samples of seed and leaves/stems (gin byproducts) were harvested 180 days after application for the PRE-T plot and 104 days after application for the POE-T plot. The seed cotton was ginned and delinted using a small-scale gin at Excel Research Services, Inc. prior to shipment to the analytical laboratory (Monsanto Environmental Services Technology Center, St. Louis, MO). The leaves and stems were shipped to PTRL West (Hercules, CA), where they were processed by grinding in a food processer in the presence of dry ice prior to shipment to the analytical laboratory. At the laboratory, the undelinted seed was further processed by cryogenic milling using a SPEX CertiPrep Freezer/Mill; no further processing was required for the gin byproducts samples. All samples were stored frozen at Monsanto (<-20 °C) prior to and after processing when not in use.

Cotton seed samples were extracted three times with hexane followed by a single ACN extraction to remove oils, after which they were extracted four times with ACN:water (40:60; v/v). Cotton gin byproducts were extracted four times with ACN:water (40:60; v/v). Following centrifugation, the ACN/water extracts of each matrix were combined and rotary evaporated to remove the ACN and part of the water. Evaporation was stopped at near dryness for gin byproducts, while seed extracts were not evaporated to dryness because of issues caused by insoluble materials. All samples were re-dissolved in water containing 0.5% formic acid and ACN (92:8, v:v) and reserved for HPLC analysis.

Separate subsamples of PRE-T and POE-T seed were also subjected to exhaustive hexane extraction for analysis of fatty acids. The samples were extracted with hexane (3x), and the

hexane extracts were combined and evaporated to near dryness. Methanol and concentrated sulfuric acid were added, and the samples were refluxed for 2.5 hours, then cooled and partitioned with hexane (3x). The combined extracts were evaporated to near dryness and reconstituted in hexane for analysis by HPLC.

The non-extractable residues remaining following ACN/water extraction of seed and gin byproducts were subjected to the following sequential enzymatic and chemical hydrolysis/oxidation procedures; mixtures were centrifuged or filtered after each step.

Both seed and gin trash:

1) Phosphate rinse (in 0.05 M phosphate buffer, pH 7.0, at RT, for 20 min.)
2) α-Amylase hydrolysis for starch (in 0.05 M phosphate buffer, pH 7.0, at 30 °C for 20 hr.)
3) Protease hydrolysis for protein (in TRIS buffer, pH 7.0, at 30 °C for 19 hr.)
4) EDTA extraction for pectin (with 0.05 M EDTA:0.05 M sodium acetate buffer, pH 5.0, at 80 °C for 5 hr., then at RT overnight)
5) Chlorite oxidation for lignin (in glacial acetic acid containing sodium chlorite at 70 °C for 1 hr., then repeated with water, acetic acid, and sodium chlorite)
6) Cellulase hydrolysis for cellulose (in 0.05 M sodium acetate buffer, pH 5.0, at 37 °C for 20 hr.)
7) 24% KOH hydrolysis for hemicellulose (from 37 to 24 °C for several hours, then at 24 °C overnight)

Seed only:

8) Concentrated sulfuric acid (at RT, overnight)
9) Protease hydrolysis (POE-T seed; in 0.05 M TRIS buffer, pH 7.0, at 30 °C for 23 hr.)
10) Hot DMSO extraction (POE-T seed; heated to 78 °C over 1.5 hr., at RT for 17 hr., heated to 70 °C over several hr., at RT for 2 hr., heated to 77 °C over 2.5 hr., cooled to RT)

Partitioning with ethyl acetate was used to investigate organic/aqueous partitioning behavior of dicamba residues and to facilitate quantitation of the metabolites or to facilitate isolation and purification of metabolites. The combined ACN/water extracts of seed and gin byproducts were rotary evaporated to remove ACN, and the remaining aqueous solution was acidified to pH 1-2 with concentrated HCl and partitioned three times with ethyl acetate. The ethyl acetate phase was evaporated to dryness, and the residue was reconstituted in 0.5% aqueous formic acid and acetonitrile for HPLC analysis. The hydrolysates resulting from protease hydrolysis of PRE-T seed and 24% KOH hydrolysis of PRE-T and POE-T seed were acidified to pH <2 with concentrated HCl, then partitioned three times with ethyl acetate. For each digest, the combined ethyl acetate extracts and the remaining aqueous layer were aliquoted for LSC analysis. Quantitative results for the partitioning procedures are not included herein.

B.4.2. Analytical Methodology

TRR were determined by combustion/LSC. Radioactivity in extracts was determined by direct LSC, and radioactivity in non-extractable fractions was determined by combustion/LSC. The limit of quantitation for TRR determinations was not reported.

Seed and gin byproducts extracts were subjected to HPLC/UV analysis for quantitation and generation of the metabolite profile. The HPLC systems were equipped with various columns, a UV detector (280, 254, or 210 nm), and a radioactive flow detector. Two methods were employed for detection and quantitation of radioactivity: HPLC with radioactive flow detection (HPLC/RAD) in which the effluent from the UV detector was pumped through the flow detector to a detection cell for an analytical scale or semi-preparative scale HPLC column, and HPLC with fraction collection and LSC detection (HPLC/LSC), in which fractions were collected at 0.3-min. intervals. HPLC Method B (C-18 column, gradient mobile phase of 0.5% formic acid and ACN) using HPLC/RAD and HPLC/LSC was the primary method used for residue quantitation and for generation of the metabolite profile.

Up to 38 metabolite peaks were observed in cotton seed and gin byproducts. All metabolite peaks accounting for ≥2% TRR were isolated and purified by preparative HPLC for further investigation. Metabolites were isolated by preparative HPLC fractionation using HPLC method (Method B). The isolated metabolite fractions were typically purified by preparative fractionation using HPLC Method D (phenyl column, gradient mobile phase of 0.5% formic acid and ACN). HPLC Method C (C18 column, gradient mobile phase of 0.5% formic acid and methanol) was used to further separate isolated metabolites from matrix contaminants.

The primary technique for identification of metabolites was mass spectral analysis which provided molecular weights and some structural information. LC/MS or LC/MS/MS with electrospray ionization (ESI) was conducted using an ion trap mass spectrometer interfaced with an Agilent 1200 RRLC system and HPLC methods M (C18 column, gradient mobile phase of 0.1% formic acid and methanol), N and O (phenyl column, gradient mobile phase of 0.1% formic acid and ACN), P (amino column, isocratic mobile phase of 0.03% NH_4OH and ACN), and Q (amino column, isocratic mobile phase of water and ACN). Typically, negative ion MS was utilized for the metabolites and their hydrolysis products, and positive ion MS was utilized for neutral derivatives of the metabolites and for the two amides (dicamba amide and DCSA amide). GC/EI/MS conducted in the positive ion mode was utilized for volatile derivatives of the metabolites or their derivatives or hydrolysis products.

HPLC co-chromatography and/or retention time comparisons, as well as comparison of the chromatographic and mass spectral properties of the metabolite derivatives or hydrolysates to those of synthetic reference standards was used to confirm the identities of the metabolites. The chemical names and structures of the reference standards used in the study are presented in Appendix I.

For the purpose of identification, metabolite peaks were isolated primarily from the POE-T gin byproducts; metabolites were not specifically isolated from the seed due to low residue levels (with the exceptions of Peaks 19, 34, and 36 from POE-T seed). It was assumed that metabolite peaks in the seed extracts represent the same metabolite peaks in the gin byproducts with the same or similar retention times. To confirm the identity of the peaks in seed, the PRE-T seed extract was co-injected with a mixture of standards corresponding to metabolites identified in POE-T and PRE-T gin byproducts, and the retention times were compared and analyzed by HPLC/RAD using Method B.

Prior to analysis by HPLC or mass spectrometry methods, isolated metabolites were characterized using a variety of methods alone or in combination to provide additional structural information, including:

- Partitioning with ethyl acetate (see above)
- Acid hydrolysis (to effect removal of sugar moieties) with 2 N HCl (at 95-100 °C for 1-2 hr.) or 1 N HCl at (60 °C for 4 hr.)
- Base hydrolysis with 2 N NaOH (at 60 °C for 4 hr.)
- Methylation (for detection of carboxylic acid and/or phenolic groups) with trimethylsilyldiazomethane at RT for 35 min.
- Acetylation (for detection of hydroxyl or phenolic groups) with acetic anhydride (at RT for 1-2 hr. or overnight)

Identification of the metabolites DCGA glucoside (Peak 6) and DCSA glucoside (Peak 19) were achieved by LC/MS and/or HPLC/RAD comparisons with the same metabolites isolated from soy forage from a metabolism study on dicamba-tolerant soybean (Report No. MSL0022659; no MRID found).

Metabolite Peak 1 (found in all matrices) was isolated from ACN/water extracts HPLC Method B. Peak 1 was also isolated from POE-T seed extracts using C18 solid phase extraction and sequential elution with water, ACN, and ethyl acetate. Peak 1 was also characterized by a number of procedures including: partitioning with ethyl acetate at acidic and neutral pH (<1.5 and 6); acid hydrolysis followed by HPLC; HPLC using Method J (amino column and isocratic mobile phase of 0.03% NH_4OH in water and ACN) to isolate possible saccharides and amino acids; and HPLC (PRE-T seed) using Method R (amino column, gradient mobile phase of water and ACN) to resolve component peaks in the organic phase following ethyl acetate partitioning. The Peak 1 radioactive components were also analyzed by negative ion electrospray LC/MS using HPLC Method Q and were compared to representative mono-, di- and tri-saccharide reference standards and amino acids. Confirmation of the identities of the mono and disaccharides observed in the LC/MS analyses was achieved by derivatization and GC/EI/MS analyses.

For identification of triglycerides, including fatty acids and glycerol in the hexane extracts of seed, the hexane phase following acidic methanolysis was analyzed by HPLC Method I (silver-impregnated strong cation exchange column, isocratic mobile phase of 1% ACN in hexane and hexanes). The peaks observed were consistent with those of the fatty acid methyl ester reference standards of stearic, oleic, and linoleic acid. Radioactivity remaining in the methanol fraction was designated glycerol.

C. RESULTS AND DISCUSSION

The sample storage conditions and durations for dicamba-tolerant cotton matrices are presented in Table C.1.1. TRR determinations were completed within 10 days of harvest. The petitioner stated that initial HPLC analyses were conducted for POE-T seed and gin trash extracts within 30 days of sample receipt to establish baseline profiles. Based on the dates of sample collection (10/28/09) and the experimental completion date (2/18/11, storage stability study), final analysis

of all samples was conducted within 478 days (15.7 months) of sample collection. To demonstrate storage stability, extracts of PRE-T and POE-T seed and gin byproducts were analyzed at the beginning of the study (47-99 days after collection), at a midway point (296-308 days after collection) and again at 475-478 days after collection (POE-T samples only). The extractability of radioactivity was similar at all time intervals (Table C.1.2), and comparison of the metabolite profiles for the POE-T seed and gin byproduct samples following initial and final analysis indicated only negligible changes in distribution of residues. These data are sufficient to support the sample storage conditions and intervals from the submitted study.

TRR in dicamba-resistant matrices are reported in Table C.2.1. TRR were determined by combustion/LSC. Following a single pre-emergence soil application of [^{14}C]dicamba at 2.094 lb ae/A, TRR were 0.1621 ppm in seed and 0.8493 ppm in gin byproducts harvested at a 180-day PHI. Following a single post-emergence foliar application at 2.065 lb ae/A, TRR were 0.9778 ppm in seed and 60.0235 ppm in gin byproducts harvested at a 104-day PHI.

The distribution of radioactivity in dicamba-tolerant cotton matrices is presented in Table C.2.2. Extraction with hexane released 18.91% and 11.47% TRR from PRE-T and POE-T seed, respectively; extraction with acetonitrile (ACN) and ACN/water together released an additional 11.91% and 26.78% TRR, respectively. In gin byproducts, extraction with ACN/water released the majority of the radioactivity: 76.25% and 71.21% TRR from PRE-T and POE-T gin byproducts, respectively. Remaining non-extractable residues for all matrices were subjected to sequential enzymatic and chemical hydrolysis and oxidation procedures, including a phosphate rinse followed by hydrolysis with α-amylase and protease; EDTA extraction; oxidation with chlorite; hydrolysis with cellulase, 24% aqueous KOH, and sulfuric acid (seed only); a second hydrolysis with protease (POE-T seed only); and hot DMSO extraction (POE-T seed only). These procedures released 52.12-61.82% TRR in seed and 21.13-28.18% TRR in gin byproducts. Non-extractable residues remaining following hydrolysis procedures were 7.35% TRR (0.012 ppm) in PRE-T seed, 9.66% TRR (0.094 ppm) in POE-T seed, 2.62% TRR (0.022 ppm) in PRE-T gin byproducts, and 0.61% TRR (0.365 ppm) in POE-T gin byproducts. These procedures adequately extracted the majority of residues (90.3-99.4%) from all cotton matrices. Extraction results were normalized; therefore, accountabilities were ~100%.

Up to 38 peaks were observed in the initial HPLC/UV profiles of seed and gin byproducts. All metabolite peaks accounting for ≥2% TRR were isolated and purified by preparative HPLC for further investigation. After isolation, residues were identified by HPLC/UV and by mass spectral analysis including LC/MS and LC/MS/MS; GC/EI/MS was used for volatile derivatives of the metabolites or their hydrolysis products. Isolated metabolites were subjected to partitioning with ethyl acetate, acid, base or enzymatic hydrolysis, and/or chemical derivatizations (methylation and acetylation) to provide additional structural information. Comparison of the chromatographic and mass spectral properties of the metabolite derivatives or hydrolysates to those of synthetic reference standards was used to confirm the identities of the metabolites.

The characterization and identification of residues in dicamba-tolerant cotton matrices are summarized in Table C.2.3. The parent, dicamba, was identified in all matrices at low levels:

0.09-0.85% TRR (0.0001-0.008 ppm) in seed and 0.50-4.48% TRR (0.004-2.691 ppm) in gin byproducts. Metabolites DCSA glucoside, DCSA, and DCGA glucoside were also present in all matrices. DCSA glucoside was the major identified metabolite in gin byproducts, at 16.83-27.77% TRR (0.236-10.101 ppm) and was present in seed at low levels (0.73-3.42% TRR). Free DCSA was the next most abundant metabolite in POE-T gin byproducts (13.39% TRR, 8.035 ppm) and was present in PRE-T gin byproducts at 5.42% TRR and in seed at 0.06-1.91%TRR. DCGA glucoside was identified at low levels in all matrices (0.09-2.46% TRR). The remaining identified metabolites were found in gin byproducts only and accounted for ≤4.71% TRR each, including MCTHBA glucoside, MCDHBA glucoside sulfate, and MCDHBA glucoside in POE-T gin byproducts, MCTHBA cyclic glucoside in both PRE-T and POE-T gin byproducts, and dicamba amide in PRE-T gin byproducts only. Because dicamba amide was only observed in PRE-T plants, it is believed to be a soil metabolite that was taken up by the plants.

Remaining extractable radioactivity in cotton seed and gin byproducts was characterized as triglycerides and sugars/natural products. In the hexane extracts of seed, triglycerides including fatty acids (palmitic, stearic, linoleic, and oleic) accounted for 10.07-16.05% TRR (0.026-0.098 ppm), while glycerol accounted for 1.35-2.86% TRR. In the ACN/water extracts of all matrices, sugars/natural products (Peak 1) accounted for 2.69-8.94% TRR (0.0074-1.613 ppm). This peak was characterized extensively, indicating that it was a mixture of polar materials, including sugars such as glucose, sucrose, and raffinose, and amino acids such as phenylalanine and glutamic acid. These sugars and amino acids are likely produced from extensive metabolism of dicamba to small molecules which are incorporated into natural plant constituents (such as carbohydrate, protein and oil) through normal plant metabolic processes. Unidentified components in oil accounted for 0.84-1.94% TRR in cotton seed; discrete unknowns in the ACN/water extracts together accounted for 0.86-15.96% TRR (0.0049-0.518 ppm) in seed and gin byproducts, with none present at >2.91% TRR.

Radioactivity in non-extractable residues was characterized by sequential enzymatic and chemical hydrolysis/oxidation. In seed, these procedures indicated that the majority of radioactivity was incorporated into hemicellulose (13.49-14.43% TRR, 0.023-0.132 ppm), protein (10.10-17.71% TRR, 0.029-0.099 ppm), and lignin (6.18-14.0% TRR, 0.01-0.136 ppm). Partitioning of the hemicellulose and protein fractions with ethyl acetate following acidification resulted in low levels of radioactivity in the organic phase, indicating that the residues are polar in nature and unlikely to be related to dicamba metabolites. Other fractions (including starch, pectin, and cellulose) individually accounted for ≤7.4% TRR. In gin byproducts, radioactivity was incorporated primarily into starch (2.73-7.05% TRR, 0.023-4.234 ppm), lignin (5.28-6.60% TRR, 0.045-3.962 ppm), and hemicellulose (2.80-5.35% TRR, 0.045-1.679 ppm). Other fractions (including protein, pectin, and cellulose) individually accounted for ≤6.2% TRR. The conclusion from these analyses is that the metabolism of dicamba to small organic molecules results in the incorporation of the label into endogenous plant components (e.g., proteins, sugars, lignin, etc.) through normal metabolic processes.

The following peaks were designated unidentified following characterization/identification attempts; however, the petitioner provided the following tentative identifications:

- Peak 5: a highly conjugated dicamba-related compound

- Peak 9: conjugate of DCSA amide (Peak 27)
- Peak 14: conjugate of DCSA amide (Peak 27), or 3-chloro-2,6-dihydroxybenzoic acid
- Peak 27: primary amide of DCSA (DCSA amide)
- Peak 32: conjugate of DCSA

C.1. Storage Stability

TRR determinations were completed within 10 days of harvest. The petitioner stated that initial HPLC analyses were conducted for POE-T seed and gin trash extracts within 30 days of sample receipt to establish baseline profiles. Based on the dates of sample collection (10/28/09) and the experimental completion date (2/18/11, storage stability study), final analysis of all samples was conducted within 478 days (15.7 months) of sample collection. To demonstrate storage stability, extracts of PRE-T and POE-T seed and gin byproducts were analyzed at the beginning of the study (47-99 days after collection), at a midway point (296-308 days after collection) and again at 475-478 days after collection (POE-T samples only). The extractability of radioactivity was similar at all time intervals (Table C.1.2), and comparison of the metabolite profiles for the POE-T seed and gin byproduct samples following initial and final analysis indicated only negligible changes in distribution of residues. These data are sufficient to support the sample storage conditions and intervals from the submitted study.

TABLE C.1.1. Summary of Storage Conditions

Matrix	Storage Temperature (°C)	Actual Study Duration[1]	Interval of Demonstrated Storage Stability
Seed	<-20	47 days	478 days (15.7 months)
Gin byproducts		79-99 days	475 days (15.6 months)

[1] Interval between harvest and initial analysis of extracts by HPLC.

TABLE C.1.2. Comparison of Extraction Results Obtained at the Beginning, Middle, and End of the Analytical phase.

Matrix	Normalized Extractability (%)		
	Initial (47-79 days)	Mid (296-308 days)	Final (475-478 days)
PRE-T Seed	27.42	30.84	Not analyzed
PRE-T Gin byproducts	73.57	76.24	
POE-T Seed	35.07	38.26	40.37
POE-T Gin byproducts	67.76	71.28	69.10

[1] Interval between harvest and initial analysis of extracts by HPLC.

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1. Total Radioactive Residues (TRR) in Dicamba-Tolerant Cotton Matrices.

Matrix	Timing and Applic. No.	PHI (days)	Residues (ppm [^{14}C]dicamba)	
			Seed	Gin byproducts
Dicamba-tolerant cotton	Single pre-emergence: 2.094 lb ae/A (2,347 g ae/ha)	180	0.1621	0.8493
	Single post-emergence: 2.065 lb ae/A (2,315 g ae/ha)	104	0.9778	60.0235

TABLE C.2.2. Distribution of the Parent and the Metabolites in Dicamba-Tolerant Cotton Matrices Following Application of [Phenyl-U-^{14}C]dicamba at 2.094 lb ae/A (pre-emergence) or 2.065 lb ae/A (post-emergence).[1]

Metabolite Fraction	Preemergence				Postemergence			
	Seed		Gin byproducts		Seed		Gin byproducts	
	TRR = 0.1621 ppm		TRR = 0.8493 ppm		TRR = 0.9778 ppm		TRR = 60.024 ppm	
	% TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Hexane [initial extracts][2]	20.25	0.0328			11.51	0.113		
Triglycerides	14.41	0.0230			8.80	0.086		
Unidentified	5.84	0.0098			2.71	0.027		
Hexane [acidic methanolysis][3]	18.91	0.031			11.47	0.112		
Hexane: Fatty acids[4]	16.05	0.026			10.07	0.098		
Acidic MeOH: Glycerol	2.86	0.005			1.35	0.013		
Unidentified (oil)	1.94	0.003			0.84	0.008		
ACN	0.44	0.001			0.48	0.005		
ACN/water	11.47	0.019	76.25	0.648	26.30	0.257	71.21	42.744
Combined ACN, ACN/water[5]	11.91	0.020			26.78	0.262		
Dicamba	0.09	0.0001	0.50	0.004	0.85	0.008	4.48	2.691
DCSA glucoside	0.73	0.0012	27.77	0.236	3.42	0.033	16.83	10.101
DCSA	0.06	0.0001	5.42	0.046	1.91	0.019	13.39	8.035
DCGA glucoside	0.09	0.0001	0.68	0.006	1.10	0.011	2.46	1.476
MCTHBA glucoside	--	--	--	--	--	--	3.33	1.998
MCDHBA glucoside sulfate	--	--	--	--	--	--	4.71	2.828
MCDHBA glucoside	--	--	--	--	--	--	0.84	0.505
MCTHBA cyc. glucoside	--	--	2.94	0.025	--	--	2.76	1.656
Dicamba amide	--	--	3.22	0.027	--	--	--	--
Sugars/natural products	4.59	0.0074	8.94	0.076	5.61	0.055	2.69	1.613
Discrete unknowns[6]	3.03	0.0049	15.96	0.136	5.36	0.052	0.86	0.518
Nonextractable	69.16	0.112	23.76	0.202	61.74	0.604	28.72	17.239
Phosphate rinse	3.87	0.006	3.25	0.028	1.96	0.019	6.20	3.72
α-Amylase (starch)	2.79	0.005	2.73	0.023	2.19	0.021	7.05	4.234
Protease (protein)	17.71	0.029	1.71	0.015	7.43	0.073	2.25	1.350
EDTA extract (pectin)	7.40	0.012	1.91	0.016	4.04	0.039	2.66	1.595
Chlorite (lignin)	6.18	0.010	5.28	0.045	8.43	0.082	6.60	3.962
Cellulase (cellulose)	4.37	0.007	0.90	0.008	3.13	0.031	0.62	0.374
24% KOH (hemicellulose)	14.43	0.023	5.35	0.045	13.49	0.132	2.80	1.679

TABLE C.2.2. Distribution of the Parent and the Metabolites in Dicamba-Tolerant Cotton Matrices Following Application of [Phenyl-U-^{14}C]dicamba at 2.094 lb ae/A (pre-emergence) or 2.065 lb ae/A (post-emergence).[1]

Metabolite Fraction	Preemergence				Postemergence			
	Seed		Gin byproducts		Seed		Gin byproducts	
	TRR = 0.1621 ppm		TRR = 0.8493 ppm		TRR = 0.9778 ppm		TRR = 60.024 ppm	
	% TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Sulfuric acid	5.07	0.008			3.21	0.031		
Protease (second protein)					2.67	0.026		
Hot DMSO (second lignin)					5.57	0.054		
Nonextractable	7.35	0.012	2.62	0.022	9.66	0.094	0.61	0.365

[1] Extraction results were normalized for the original extraction procedures and for characterization of nonextractable residues (See Tables 14 and 15). Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
[2] Results as reported in Tables 9 and 11.
[3] Calculated by the study reviewer to show distribution of triglycerides as fatty acids and glycerol, based on normalized values reported in Table 13.
[4] Including palmitic, stearic, oleic, and linoleic acids
[5] Calculated by the study reviewer by summing.
[6] Unknowns (discrete quantitated peaks) are comprised of 19 components in PRE-T seed (each ≤0.45% TRR, ≤0.0007 ppm); 12 components in POE-T seed (each ≤0.81% TRR, ≤0.008 ppm); 11 components in PRE-T gin byproducts (each ≤2.91% TRR, ≤0.025 ppm); and 1 component in POE-T gin byproducts (0.86% TRR, 0.518 ppm).

TABLE C.2.3. Summary of Characterization and Identification of Radioactive Residues in Dicamba-Tolerant Cotton Matrices Following Application of [Phenyl-U-^{14}C]dicamba at 2.094 lb ae/A (pre-emergence) or 2.065 lb ae/A (post-emergence)

	Preemergence				Postemergence			
	Seed		Gin byproducts		Seed		Gin byproducts	
	TRR = 0.1621 ppm		TRR = 0.8493 ppm		TRR = 0.9778 ppm		TRR = 60.024 ppm	
	% TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Dicamba	0.09	0.0001	0.50	0.004	0.85	0.008	4.48	2.691
DCSA glucoside	0.73	0.0012	27.77	0.236	3.42	0.033	16.83	10.101
DCSA	0.06	0.0001	5.42	0.046	1.91	0.019	13.39	8.035
DCGA glucoside	0.09	0.0001	0.68	0.006	1.1	0.011	2.46	1.476
MCTHBA glucoside	--	--	--	--	--	--	3.33	1.998
MCDHBA glucoside sulfate	--	--	--	--	--	--	4.71	2.828
MCDHBA glucoside	--	--	--	--	--	--	0.84	0.505
MCTHBA cyc. glucoside	--	--	2.94	0.025	--	--	2.76	1.656
Dicamba amide	--	--	3.22	0.027	--	--	--	--
Fatty acids	16.05	0.026	--	--	10.07	0.098	--	--
Glycerol	2.86	0.005	--	--	1.35	0.013	--	--
Sugars/natural products	4.59	0.0074	8.94	0.076	5.61	0.055	2.69	1.613
Phosphate rinse	3.87	0.006	3.25	0.028	1.96	0.019	6.20	3.72
Starch	2.79	0.005	2.73	0.023	2.19	0.021	7.05	4.234
Protein	17.71	0.029	1.71	0.015	10.10	0.099	2.25	1.350
Pectin	7.40	0.012	1.91	0.016	4.04	0.039	2.66	1.595

TABLE C.2.3. Summary of Characterization and Identification of Radioactive Residues in Dicamba-Tolerant Cotton Matrices Following Application of [Phenyl-U-^{14}C]dicamba at 2.094 lb ae/A (pre-emergence) or 2.065 lb ae/A (post-emergence)

	Preemergence				Postemergence			
	Seed		Gin byproducts		Seed		Gin byproducts	
	TRR = 0.1621 ppm		TRR = 0.8493 ppm		TRR = 0.9778 ppm		TRR = 60.024 ppm	
	% TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Lignin	6.18	0.010	5.28	0.045	14.00	0.136	6.60	3.962
Cellulose	4.37	0.007	0.90	0.008	3.13	0.031	0.62	0.374
Hemicellulose	14.43	0.023	5.35	0.045	13.49	0.132	2.80	1.679
Acid Hydrolysates	5.07	0.008	--	--	3.21	0.031	--	--
Unidentified (oil)	1.94	0.003	--	--	0.84	0.008	--	--
Discrete unknowns	3.03	0.0049	15.96	0.136	5.36	0.052	0.86	0.518
Total identified	0.97	0.0015	40.53	0.344	7.28	0.071	48.80	29.29
Total characterized	90.29	0.1463	46.03	0.392	75.35	0.734	31.73	19.045
Total extractable	92.65	0.150	97.38	0.827	90.34	0.883	99.39	59.659
Unextractable (PES)[1]	7.35	0.012	2.62	0.022	9.66	0.094	0.61	0.365
Accountability[2]	100		100		100		100	

[1] Residues remaining after exhaustive extractions.
[2] Accountability = (Total extractable + Total unextractable)/(TRR from combustion analysis; see TABLE C.2.1) * 100.

C.3. Proposed Metabolic Profile

Based on the results of the metabolism study on dicamba-tolerant cotton, the petitioner proposes that the metabolism of dicamba proceeds by initial demethylation of dicamba to form DCSA through the action of the dicamba *O*-demethylase enzyme, which is the product of the DMO gene introduced to confer dicamba tolerance. The petitioner noted that, due to the predominance of the *O*-demethylation pathway, 5-OH-dicamba is not observed as a metabolite in dicamba-tolerant cotton or soybean. While free DCSA was observed in cotton matrices (POE-T and PRE-T gin byproducts in particular), the majority of DCSA is found as its conjugate DCSA glucoside. Some DCSA glucoside is further modified by hydroxyl replacement of chlorine to form MCDHBA glucoside which is then further conjugated by sulfation to form MCDHBA glucoside sulfate. In a minor pathway, DCSA is hydroxylated at the 5-position, presumably by a P-450 enzyme or other oxygenase, to form DCGA. DCGA is not observed as a free metabolite in cotton matrices but is converted to DCGA glucoside in which the glucose moiety is attached to the hydroxyl group at the 5-position of DCGA (based upon comparison to the same metabolite identified in dicamba-tolerant soybean). DCGA glucoside is further converted to MCTHBA glucoside by hydroxyl replacement of chlorine at the 3-position. Alternatively, MCTHBA cyclic glucoside may form from DCGA glucoside through displacement of chlorine at the 6-position of the DCGA portion of the molecule by a hydroxyl group of the glucose moiety. Dicamba amide was a minor metabolite that was only observed in PRE-T matrices. The petitioner concluded that this metabolite is most likely the result of the metabolic conversion of the carboxylic acid moiety of dicamba to an amide functional moiety *via* soil microbial metabolism.

The proposed metabolic pathway is depicted in Figure C.3.1, which was copied without alteration from MRID 48728701.

FIGURE C.3.1. Proposed Metabolic Profile of Dicamba in Dicamba-Tolerant Cotton

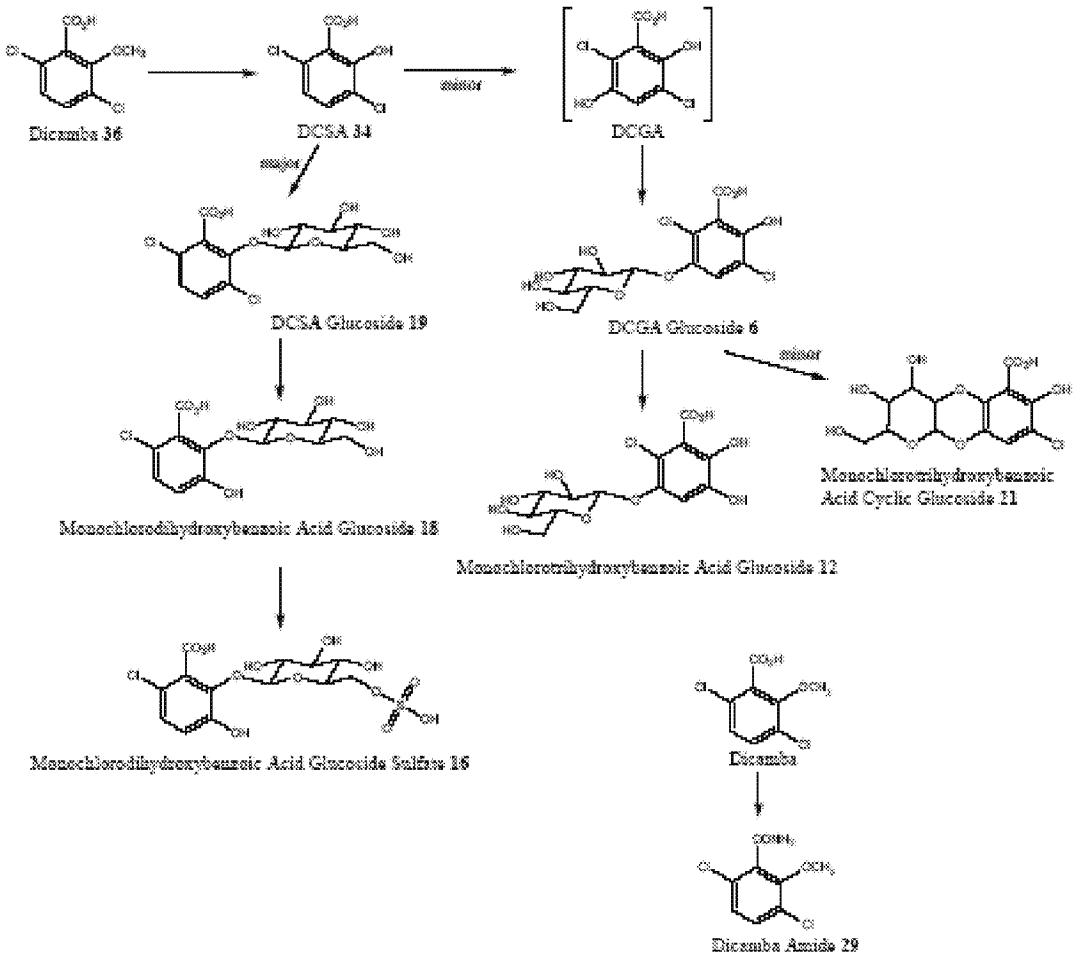


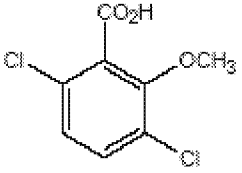
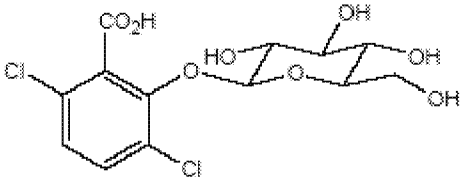
TABLE C.3.1. Identification of Compounds from Metabolism Study		
Common name/code	Chemical name	Chemical structure
Dicamba (Peak 36)	3,6-dichloro-2-methoxybenzoic acid	
DCSA Glucoside (Peak 19)	3,6-dichloro-2-(β-D-glucopyranosyloxy)benzoic acid	

TABLE C.3.1. Identification of Compounds from Metabolism Study

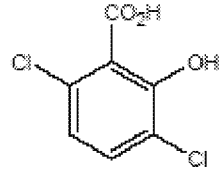
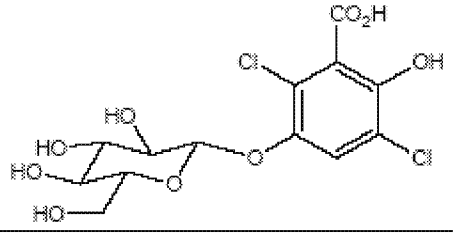
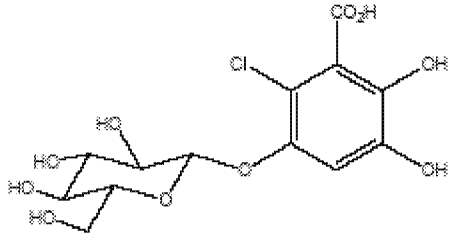
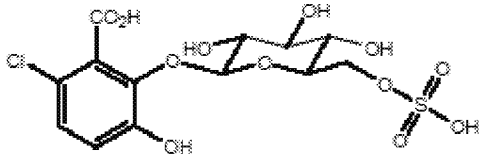
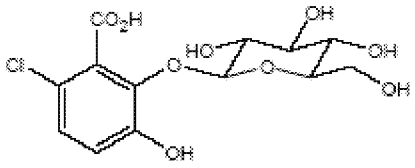
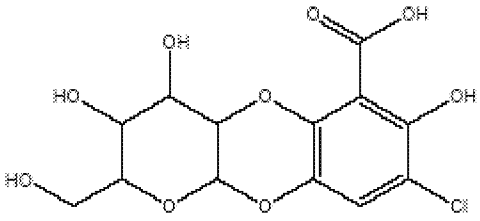
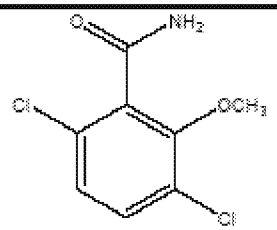
Common name/code	Chemical name	Chemical structure
DCSA (Peak 34)	3,6-dichloro-2-hydroxy benzoic acid	
DCGA Glucoside (Peak 6)	2,5-dichloro-3-(β-D-glucopyranosyloxy)-6-hydroxybenzoic acid	
MCTHBA Glucoside (Peak 12)	2-Chloro-5,6-dihydroxy-3-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydropyran-2-yloxy)benzoic acid	
MCDHBA Glucoside Sulfate (Peak 16)	6-Chloro-3-hydroxy-2-(3,4,5-trihydroxy-6-sulfooxymethyltetrahydro-pyran-2-yloxy)benzoic acid	
MCDHBA Glucoside (Peak 18)	6-Chloro-3-hydroxy-2-(3,4,5-trihydroxy-6-hydroxymethyltetrahydro-pyran-2-yloxy)benzoic acid	
MCTHBA Cyclic Glucoside (Peak 21)	7-Chloro-3,4,6-trihydroxy-2-hydroxymethyl-3,4,4a,9a-tetrahydro-2*H*-1,9.10-trioxa-anthracene-5-carboxylic acid	

TABLE C.3.1. Identification of Compounds from Metabolism Study		
Common name/code	Chemical name	Chemical structure
Dicamba Amide (Peak 29)	3,6-dichloro-2-methoxybenzamide	

D. CONCLUSION

The results of the metabolism study adequately delineate the nature of the residue in dicamba-tolerant cotton treated with dicamba. Following a single pre-emergence soil application of [^{14}C]dicamba at 2.094 lb ae/A, TRR were 0.1621 ppm in seed and 0.8493 ppm in gin byproducts harvested at a 180-day PHI. Following a single post-emergence foliar application at 2.065 lb ae/A, TRR were 0.9778 ppm in seed and 60.0235 ppm in gin byproducts harvested at a 104-day PHI.

The parent, dicamba, was identified in all matrices at low levels: 0.09-0.85% TRR in seed and 0.50-4.48% TRR in gin byproducts. Metabolites DCSA glucoside, DCSA, and DCGA glucoside were also present in all matrices. DCSA glucoside was the major identified metabolite in gin byproducts, at 16.83-27.77% TRR and was present in seed at low levels (0.73-3.42% TRR). Free DCSA was the next most abundant metabolite in POE-T gin byproducts (13.39% TRR) and was present in PRE-T gin byproducts at 5.42% TRR and in seed at 0.06-1.91%TRR. DCGA glucoside was identified at low levels in all matrices (0.09-2.46% TRR). The remaining identified metabolites were found in gin byproducts only and accounted for ≤4.71% TRR each, including MCTHBA glucoside, MCDHBA glucoside sulfate, and MCDHBA glucoside in POE-T gin byproducts, MCTHBA cyclic glucoside in both PRE-T and POE-T gin byproducts, and dicamba amide in PRE-T gin byproducts only. Because dicamba amide was only observed in PRE-T plants, it is believed to be a soil metabolite that was taken up by the plants.

Remaining extractable radioactivity in seed and gin byproducts was characterized as triglycerides and sugars/natural products. In the hexane extracts of seed, triglycerides including fatty acids (palmitic, stearic, linoleic, and oleic) accounted for 10.07-16.05% TRR, while glycerol accounted for 1.35-2.86% TRR. In the ACN/water extracts of all matrices, sugars/natural products (Peak 1) accounted for 2.69-8.94% TRR. Unidentified components in oil accounted for 0.84-1.94% TRR in seed; discrete unknowns in the ACN/water extracts together accounted for 0.86-15.96% TRR in seed and gin byproducts, with none present at >2.91% TRR. Radioactivity in non-extractable residues was characterized by sequential enzymatic and chemical hydrolysis/oxidation. In seed, these procedures indicated that the majority of radioactivity was incorporated into hemicellulose (13.49-14.43% TRR), protein (10.10-17.71% TRR), and lignin (6.18-14.0% TRR). Other fractions (including starch, pectin, and cellulose) individually accounted for ≤7.4% TRR. In gin byproducts, radioactivity was incorporated primarily into starch (2.73-7.05% TRR), lignin (5.28-6.60% TRR), and hemicellulose (2.80-

5.35% TRR). Other fractions (including protein, pectin, and cellulose) individually accounted for ≤6.2% TRR.

Based on the results of the metabolism study on dicamba-tolerant cotton, the petitioner proposes that the metabolism of dicamba proceeds by initial demethylation of dicamba to form DCSA through the action of the dicamba *O*-demethylase enzyme, which is the product of the DMO gene introduced to confer dicamba tolerance. The petitioner noted that, due to the predominance of the *O*-demethylation pathway, 5-OH-dicamba is not observed as a metabolite in dicamba-tolerant cotton or soybean. While free DCSA was observed in cotton matrices (POE-T and PRE-T gin byproducts in particular), the majority of DCSA is found as its conjugate DCSA glucoside. Some DCSA glucoside is further modified by hydroxyl replacement of chlorine to form MCDHBA glucoside which is then further conjugated by sulfation to form MCDHBA glucoside sulfate. In a minor pathway, DCSA is hydroxylated at the 5-position, presumably by a P-450 enzyme or other oxygenase, to form DCGA. DCGA is not observed as a free metabolite in cotton matrices but is converted to DCGA glucoside in which the glucose moiety is attached to the hydroxyl group at the 5-position of DCGA (based upon comparison to the same metabolite identified in dicamba-tolerant soybean). DCGA glucoside is further converted to MCTHBA glucoside by hydroxyl replacement of chlorine at the 3-position. Alternatively, MCTHBA cyclic glucoside may form from DCGA glucoside through displacement of chlorine at the 6-position of the DCGA portion of the molecule by a hydroxyl group of the glucose moiety. Dicamba amide was a minor metabolite that was only observed in PRE-T matrices. The petitioner concluded that this metabolite is most likely the result of the metabolic conversion of the carboxylic acid moiety of dicamba to an amide functional moiety *via* soil microbial metabolism.

E. REFERENCES

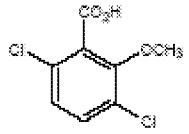
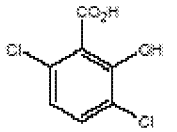
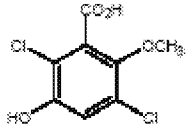
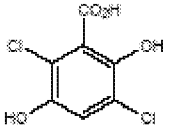
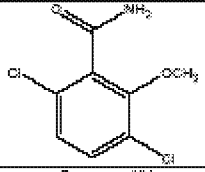
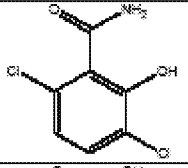
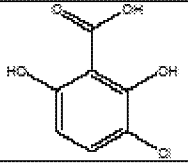
None.

F. DOCUMENT TRACKING

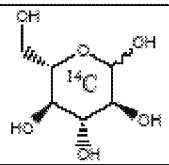
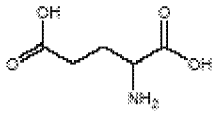
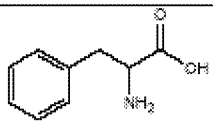
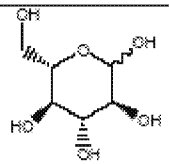
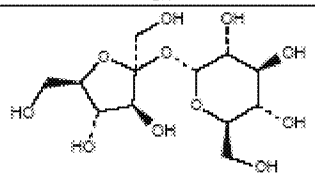
Petition Number(s): 2F8067
DP Barcode(s): 408384
PC Code: 029801, 029802, 029806, 128931, 128944, & 129043

Template Version June 2005.

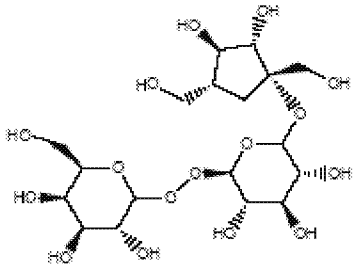
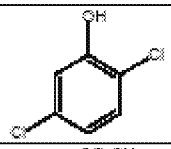
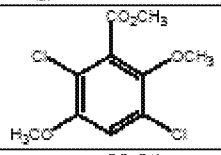
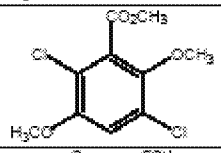
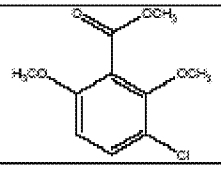
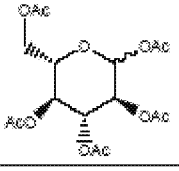
APPENDIX I. Chemical Names and Structures of Reference Standards Used in Cotton Metabolism Study.

Reference Number	Trivial Name and Chemical Name	Structure
I	Dicamba 3,6-dichloro-2-methoxy benzoic acid	
II	DCSA 3,6-dichloro-2-hydroxy benzoic acid	
III	5-Hydroxydicamba 2,5-dichloro-3-hydroxy-6-methoxybenzoic acid	
IV	DCGA 2,5-dichloro-3,6-dihydroxybenzoic acid	
V	Dicamba Amide 3,6-dichloro-2-methoxy benzamide	
VI	DCSA Amide 3,6-dichloro-2-hydroxy benzamide	
VII	CDHBA 3-Chloro-2,6-dihydroxybenzoic Acid	
VIII	Palmitic Acid, Methyl Ester hexadecanoic acid, methyl ester	

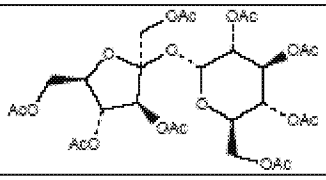
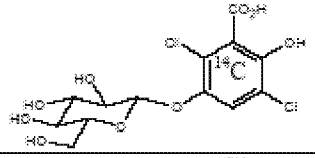
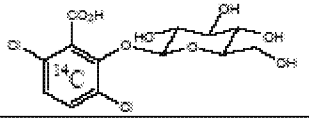
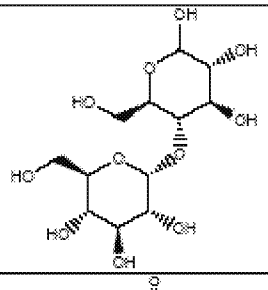
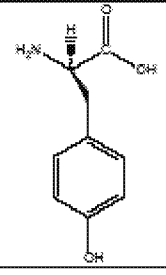
APPENDIX I. Chemical Names and Structures of Reference Standards Used in Cotton Metabolism Study (cont.).

Reference Number	Trivial Name and Chemical Name	Structure
IX	Stearic acid, Methyl Ester octadecanoic acid, methyl ester	
X	Oleic Acid, Methyl Ester 9-octadecenoic acid (9Z)-, methyl ester	
XI	Linoleic Acid, Methyl Ester 9,12-octadecadienoic acid (9Z,12Z)-, methyl ester	
XII	^{14}C-D-Glucose 6-hydroxymethyl-tetrahydro-pyran-2,3,4,5-tetraol	
XIII	L-Glutamic Acid 2-amino-pentanedioic acid	
XIV	L-Phenylalanine 2-amino-3-phenyl-propionic acid	
XV	α-D-Glucose 6-hydroxymethyl-tetrahydro-pyran-2,3,4,5-tetraol	
XVI	Sucrose β-D-fructofuranosyl-(2→1)-α-D-glucopyranoside	

APPENDIX I. Chemical Names and Structures of Reference Standards Used in Cotton Metabolism Study (cont.).

Reference Number	Trivial Name and Chemical Name	Structure
XVII	D-(+)-Raffinose pentahydrate	
XVIII	2,5-Dichlorophenol	
XIX	Trimethyl DCGA 2,5-dichloro-3,6-dimethoxy benzoic acid, methyl ester	
XX	Trimethyl DCGA 2,5-dichloro-3,6-dimethoxy benzoic acid, methyl ester	
XXI	Trimethyl CDHBA 3-Chloro-2,6-dimethoxy-benzoic acid, methyl ester	
XXII	Pentaacetylglucose 3,4,5-triacetoxy-6-acetoxymethyl-tetrahydro-pyran-2-yl ester	

APPENDIX I. Chemical Names and Structures of Reference Standards Used in Cotton Metabolism Study (cont.).

Reference Number	Trivial Name and Chemical Name	Structure
XXIII	Octaacetylsucrose	
XXIV	^{14}C-DCGA Glucoside	
XXV	^{14}C-DCSA Glucoside	
XXVI	Starch from corn	Complex
XXVII	α-Cellulose	Complex
XXVIII	Casein	Complex
XXIX	D-(+)-Maltose	
XXX	L-Tyrosine	

Primary Evaluator		Date: 11/15/2012
	Versar, Inc.	
Approved by	Peter Savoia	Date: 04/22/2013
	Peter Savoia, Chemist, OCSPP-HED-RAB V/VII	

Note: This Data Evaluation Record (DER) was originally prepared under contract by Versar, Inc. (6850 Versar Center, Springfield, VA 22151; submitted 11/15/12). The DER has been reviewed by HED and revised to reflect current Office of Pesticide Programs (OPP) policies.

STUDY REPORTS:

48728702 Foster, J.; Mierkowski, M.; Miller, M. (2011) Amended Report for MSL0023267: Analytical Method for the Determination of Dicamba and its Major Metabolites in Cotton Matrices by LC/MS/MS. Project Number: AG/ME/1381/01/OCR, MSL0023759. Unpublished study prepared by Monsanto Company. 93p.

EXECUTIVE SUMMARY:

Monsanto has submitted an analytical method description and validation data for a data collection method, AG-ME-1381-01, for the determination of residues of dicamba and its metabolites, 5-hydroxydicamba (5-OH dicamba), 3,6-dichlorosalicylic acid (DCSA) and 3,6-dichlorogentisic acid (DCGA) in cotton matrices using high performance liquid chromatography with triple-quadrople mass spectrometric (LC-MS/MS) analysis. The method was used for data collection in the cotton crop field trial, processing, and storage stability studies reviewed under DP# 404821.

Briefly, samples were extracted with acetonitrile:water (40:60, v:v). An aliquot of the extract was hydrolyzed in 1 N HCl at 95 °C in an oven. The hydrolysate was partitioned with ethyl acetate:isooctane (20:80, v:v). Water was added to the organic phase and the sample was concentrated by evaporation until only the aqueous solution remained. The samples were acidified and analyzed using LC/MS/MS. Stable-labeled internal standards for each analyte ($^{13}C_6$-Dicamba, $^{13}C_6$-5-Hydroxydicamba, $^{13}C_6$-DCSA, and $^{13}C_6$-DCGA) were used to normalize for matrix effects and procedural recovery. The method monitors a single ion transition for each analyte.

The limit of quantitation (LOQ), determined as the lowest limit of method validation (LLMV), for dicamba, 5-OH dicamba, DCSA and DCGA was 0.02 ppm for each analyte in undelinted cotton seed, meal, hulls, and alkali refined oil; and 0.04 ppm for gin byproducts. The limit of detection (LOD) in cotton seed, statistically determined from the LLMV, was 0.0089, 0.0057, 0.0035, and 0.0041 ppm for dicamba, 5-OH dicamba, DCSA and DCGA, respectively. LODs were not determined for cotton gin byproduct or processed commodities.

The method was successfully validated using samples of undelinted cotton seed, meal, hulls, and oil fortified with each analyte at 0.02, 0.20, and 10 ppm; and cotton gin byproducts fortified with

each analyte at 0.04, 0.40, and 10 ppm. Mean recoveries were within the acceptable range of 70% to 120% with the coefficient of variation less than 20% for each matrix tested.

No confirmatory analysis procedures were included for the method, and no interference study was submitted; however, because the method has not been proposed for enforcement, confirmatory analysis procedures are not required.

An independent laboratory validation (ILV) was not submitted and is not required in support of a data collection method.

Monsanto submitted radiovalidation data to evaluate the efficiency of the extraction methodology presented in Method AG-ME-1381-01. Average extractabilities from gin byproduct and cotton seed using Method AG-ME-1381-01 extraction procedures (67.07% and 24.08%), were similar to normalized extractabilities obtained from gin byproduct and cotton seed samples in the metabolism study (MRID 48728701) using multiple acetonitrile:water extractions (67.76% and 24.97%). Additionally, residue levels of dicamba, DCGA, and DCSA in the acidic post-evaporation solutions obtained using LC/MS/MS method AG-ME-1381-01, were similar to the residue levels in the metabolism samples as determined by ^{14}C-HPLC profiles of hydrolyzed extracts. The extraction efficiencies for dicamba, DCGA, and DCSA, respectively, as determined by the reviewer, were 106%, 101%, and 121% in the undelinted seed samples, and 83%, 79%, and 87% in the gin byproduct samples. Monsanto also investigated the recovery of radioactivity through the post-extraction steps of method AG-ME-1381-01. Overall post-extraction recovery was 31.31% in undelinted cotton seed and 50.20% in cotton gin byproducts. The majority of the loss was in the partitioning step.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, analytical method test data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document D408384.

COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Dicamba (3,6-dichloro-2-methoxybenzoic acid) is a selective benzoic acid herbicide registered for the control of weeds prior to their emergence. The dicamba Reregistration Eligibility Decision (RED) was issued December 2005. The chemical structure and nomenclature of dicamba and its metabolites 5-OH dicamba, DSCA, and DCGA are presented in Table A.1. The physicochemical properties of the technical grade of dicamba acid are presented in Table A.2.

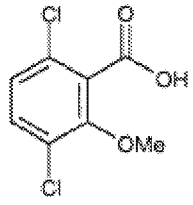
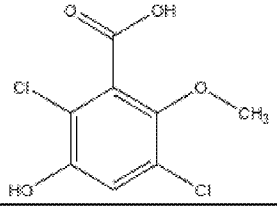
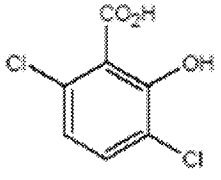
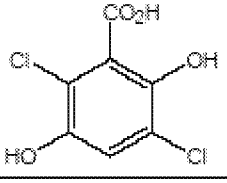
TABLE A.1. Test Compound Nomenclature.	
Compound	
Common name	Dicamba
Company experimental name	MON 11900
IUPAC name	3,6-dichloro-o-anisic acid or 3,6-dichloro-2-methoxybenzoic acid
CAS name	3,6-dichloro-2-methoxybenzoic acid
CAS registry number	1918-00-9 (dicamba acid), 104040-79-1 (diglycolamine salt), or 53404-28-7 (monoethanolamine salt)
End-use product	Not applicable
Compound	
Common name	5-Hydroxy-dicamba
Company experimental name	5-OH dicamba
IUPAC/CAS name	2,5-dichloro-3-hydroxy-6-methoxybenzoic acid
CAS registry number	7600-50-2
Compound	
Common name	DCSA; 3,6-dichlorosalicylic acid
Company experimental name	MON 52708
IUPAC/CAS name	3,6-dichloro-2-hydroxybenzoic acid
CAS registry number	3401-80-7
Compound	
Common name	DCGA; 3,6-dichlorogentistic acid
Company experimental name	MON 52724
IUPAC/CAS name	2,5-dichloro-3,6-dihydroxybenzoic acid
CAS registry number	18688-01-2

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound: Dicamba.		
Parameter	Value	Reference
Melting point	114-116 EC (PAI) 90-100 EC (87% TGAI)	Residue Chemistry Chapter of the Dicamba RED, DP# 317699, 12/20/05, C. Olinger
pH	2.5-3.0 (87% TGAI)	
Density	1.57 g/mL at 25 EC (87% TGAI)	
Water solubility	0.5 g/100 mL at 25 EC (PAI)	
Solvent solubility	g/100 mL at 25 EC (PAI) dioxane 118.0 ethanol 92.2 isopropyl alcohol 76.0 methylene chloride 26.0 acetone 17.0 toluene 13.0 xylene 7.8 heavy aromatic naphthalene 5.2	
Vapor pressure	3.4 x 10^{-5} mm Hg at 25 EC (PAI)	
Dissociation constant, pK_a	1.97 (PAI)	
Octanol/water partition coefficient, Log(K_{OW})	0.1 (PAI)	
UV/visible absorption spectrum	neutral: 511 (275 nm) acidic (pH 0-1): 1053 (281 nm) basic (pH 13-14): 469 (274 nm)	

B. MATERIALS AND METHODS

B.1. Data-Gathering Method

Monsanto has submitted an analytical method description and validation data for a data collection method, AG-ME-1381-01, for the determination of residues of dicamba and its metabolites, 5-OH dicamba, DCSA and DCGA in cotton matrices using LC/MS/MS analysis.

B.1.1. Principle of the Method:

Briefly, samples were extracted with acetonitrile:water (40:60, v:v). An aliquot of the extract was hydrolyzed in 1 N HCl at 95 °C in an oven. The hydrolysate was partitioned with ethyl acetate:isooctane (20:80, v:v). Water was added to the organic phase and the sample was concentrated by evaporation until only the aqueous solution remained. The samples were acidified and analyzed using LC/MS/MS. Stable-labeled internal standards for each analyte ($^{13}C_6$-Dicamba, $^{13}C_6$-5-Hydroxydicamba, $^{13}C_6$-DCSA, and $^{13}C_6$-DCGA) were used to normalize for matrix effects and procedural recovery. The method monitors a single ion transition for each analyte.

TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of the Residues of Dicamba and Dicamba Metabolites in Cotton Matrices.	
Method ID	AG-ME-1381-01
Analyte(s)	Dicamba, 5-OH dicamba, DCSA and DCGA
Extraction solvent/technique	Cotton matrices were extracted with acetonitrile (ACN)/water (40:60, v:v).

TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of the Residues of Dicamba and Dicamba Metabolites in Cotton Matrices.	
Cleanup strategies	An aliquot of the extract was hydrolyzed in 1 N HCl at 95 °C in an oven. The hydrolysate was partitioned with ethyl acetate:isooctane (20:80, v:v). Water was added to the organic phase and the sample was concentrated by evaporation until only the aqueous solution remained. The sample was acidified and analyzed by LC/MS/MS.
Instrument/Detector	LC/MS/MS using a Betasil Phenyl column. Quantitation of residues is performed using MS/MS turbo ion spray ionization in the negative ion MRM mode. The following ions are monitored: Dicamba: 219→175 m/z 5-OH dicamba: 237→193 m/z DCSA: 205→161 m/z DCGA: 221→177 m/z Internal Standards $^{13}C_6$-Dicamba: 225→181 m/z $^{13}C_6$-5-Hydroxydicamba: 243→199 m/z $^{13}C_6$-DCSA: 211→167 m/z $^{13}C_6$-DCGA: 227→183 m/z
Standardization method	Internal ($^{13}C_6$-Dicamba, $^{13}C_6$-5-Hydroxydicamba, $^{13}C_6$-DCSA, and $^{13}C_6$-DCGA) and external standardization using mixed standards was used. A calibration curve is constructed where the peak area is plotted versus the analyte concentration using linear regression and a correlation coefficient of 0.990 or greater is obtained.
Stability of std solutions	Stock solutions (100 µg/ml) were reported to be stable for at least 201 days. Calibration standards (0.005 to 0.500 µg/mL) were reported to be stable for dicamba, 5-OH dicamba and DCSA at the 0.005 µg/mL concentration and above for at least 175 days and for DCGA at the 0.010 µg/mL concentration and above for at least 32 days.
Retention times (minutes)	Dicamba: 2.7 5-OH dicamba: 1.3 DCSA: 5.6 DCGA: 1.7

B.2. Enforcement Method

A gas chromatography method with electron capture detection (GC/ECD) is the current enforcement method for residues of dicamba in/on plant commodities (Method AM-0691B-0297-4).

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method

Method validation data are presented in Table C.1.1. Acceptable method validation recoveries for method AG-ME-1381-01 were obtained for all analytes from samples undelinted cotton seed at 0.02, 0.20, and 10 ppm; cotton meal, hulls, and oil at 0.02 and 0.20 ppm; and cotton gin byproducts at 0.04, 0.40, and 10 ppm. Mean recoveries were within the acceptable range of 70% to 120% with the coefficient of variation less than 20% for each matrix tested. Apparent residues of dicamba and its metabolites, 5-OH dicamba, DCSA and DCGA were each < LLMV in/on two samples each of seed (<0.02 ppm), gin byproducts (<0.04 ppm), meal, hulls, and alkali refined oil (<0.02 ppm). The fortification levels used in method validation are adequate to bracket expected residue levels in/on cotton matrices.

The method characteristics for AG-ME-1381-01 are summarized in Table C.1.2. The LOQ, determined as the LLMV, was 0.02 ppm for each analyte in undelinted cotton seed, meal, hulls, and alkali refined oil; and 0.04 ppm for each analyte in gin byproducts. The LOD in cotton seed, statistically determined from the LLMV, was 0.0089, 0.0057, 0.0035, and 0.0041 ppm for dicamba, 5-OH dicamba, DCSA and DCGA, respectively. LODs were not determined for cotton gin byproduct or processed commodities.

No confirmatory analysis procedures were included for the method, and no interference study was submitted; however, because the method has not been proposed for enforcement, confirmatory analysis procedures are not required.

Monsanto also submitted radiovalidation data. In the first part of the study, Monsanto compared the amount of radioactivity extracted from cotton seed and gin byproduct samples obtained from the metabolism study (MRID 48728701) using multiple acetonitrile:water extractions, to the amount of radioactivity extracted using the analytical method AG-ME-1381-01 extraction procedures. The extractabilities were similar: for gin byproducts and undelinted cotton seed, respectively, normalized extractabilities from the metabolism study were 67.76% and 24.97% and average extractabilities from method AG-ME-1381-01 were 67.07% and 24.08%.

In the second part of the radiovalidation study, Monsanto investigated the recovery of radioactivity through the post-extraction steps of method AG-ME-1381-01. Overall post-extraction recovery was 31.31% in undelinted cotton seed and 50.20% in cotton gin byproducts. The majority of the loss was in the partitioning step. Average percent recoveries for the hydrolysis, organic partition, aqueous partition, and acidic post-evaporation steps, respectively, were 87.06%, 57.21%, 62.98%, and 62.87% in cotton seed and 82.65%, 67.98%, 39.10%, and 89.34% in gin byproducts. The study notes that losses in the partitioning step may be due to 1) water solubility of a percentage of the residues (e.g., polar residues formed by degradation of dicamba to small molecules and incorporation into natural products such as sugars), especially for seed, and, 2) incomplete partitioning of the analytes into the organic phase. The study also notes that the use of internal standards in this method compensates for any procedural losses incurred in the hydrolysis, partitioning, and evaporation steps.

In the third part of the radiovalidation study Monsanto compared the residue quantitation results of the acidic post-evaporation solutions obtained using LC/MS/MS method AG-ME-1381-01, with the actual residue levels in the metabolism samples as determined by ^{14}C-HPLC profiles of hydrolyzed extracts (MRID 48728701). These results are presented in Table C.1.3. The extraction efficiencies for dicamba, DCGA, and DCSA, respectively, were determined by the reviewer to be 106%, 101%, and 121% in the undelinted seed samples, and 83%, 79%, and 87% in the gin byproduct samples.

TABLE C.1.1 Recovery Results from Method Validation of Cotton Matrices using the Data-Gathering Analytical Method. Standards were prepared in acetonitrile.

Matrix	Spiking Level (ppm)	Recoveries Obtained[1] (%)	Mean Recovery ± SD (CV)[1] (%)
Dicamba			
Cotton seed	0.005	187, 73, 172, 105, 102	128 ± 49 (38)

TABLE C.1.1 Recovery Results from Method Validation of Cotton Matrices using the Data-Gathering Analytical Method. Standards were prepared in acetonitrile.

Matrix	Spiking Level (ppm)	Recoveries Obtained[1] (%)	Mean Recovery ± SD (CV)[1] (%)
	0.01	129, 149, 93, 78, 125	115 ± 29 (25)
	0.02	119, 100, 123, 101, 124	113 ± 12 (10)
	0.20	94.0, 96.0, 94.5, 96.5, 99.5	96.1 ± 2.2 (2.2)
	10	68.3, 90.5, 90.6, 100, 98.4	89.6 ± 13 (14)
Cotton gin byproducts	0.04	99.8, 92.5, 88.0, 122, 119	104 ± 15 (15)
	0.40	97.0, 98.5, 101, 97.5, 104	99.5 ± 2.7 (2.7)
	10	87.8, 90.5, 82.4, 85.1, 87.8	86.7 ± 3.1 (3.5)
Cotton meal	0.02	102, 101, 98.0, 111, 108	104 ± 5.3 (5.1)
	0.20	96.5, 98.5, 95.5, 100, 94.0	96.9 ± 2.4 (2.5)
Cotton hulls	0.02	96.5, 100, 106, 99.0, 98.5	100 ± 3.4 (3.4)
	0.20	96.5, 101, 102, 100, 99.0	100 ± 2.1 (2.1)
Cotton alkali refined oil	0.02	99.0, 96.0, 97.0, 98.5, 97.5	97.6 ± 1.2 (1.2)
	0.20	99.0, 100, 105, 98.5, 97.0	100 ± 2.8 (2.9)
5-OH dicamba			
Cotton seed	0.005	68, 56, 101, 84, 105	82.9 ± 21 (25)
	0.01	106, 97, 76, 110, 82	94.1 ± 15 (16)
	0.02	96.0, 97.5, 115, 117, 114	108 ± 10 (9.3)
	0.20	97.5, 104, 105, 104, 101	102 ± 3.0 (2.9)
	10	69.1, 89.6, 79.4, 93.2, 104	87.1 ± 13 (15)
Cotton gin byproducts	0.04	124, 106, 98.0, 96.5, 108	106 ± 11 (10)
	0.40	111, 113, 106, 116, 109	111 ± 3.6 (3.3)
	10	113, 98.7, 99.4, 101, 84.7	99.4 ± 10 (10)
Cotton meal	0.02	72.0, 108, 92.0, 77.0, 91.5	88.1 ± 14 (16)
	0.20	98.0, 93.5, 87.5, 98.5, 89.5	93.4 ± 4.9 (5.3)
Cotton hulls	0.02	86.5, 78.0, 106, 89.0, 97.0	91.2 ± 10 (11)
	0.20	114, 107, 117, 98.5, 105	108 ± 7.4 (6.8)
Cotton alkali refined oil	0.02	94.5, 101, 79.0, 92.0, 88.0	90.8 ± 8.0 (8.8)
	0.20	110, 103, 88.0, 110, 100	102 ± 9.0 (8.8)
DCGA			
Cotton seed	0.005	140, 115, 88, 98, 129	114 ± 22 (19)
	0.01	90, 108, 96, 79, 104	95.6 ± 11 (12)
	0.02	81.0, 98.0, 92.5, 77.0, 86.0	86.9 ± 8.5 (9.8)
	0.20	90.0, 87.0, 88.0, 84.0, 95.5	88.9 ± 4.3 (4.8)
	10	73.4, 95.7, 89.2, 94.4, 90.4	88.6 ± 8.9 (10)
Cotton gin byproducts	0.04	66.8, 87.5, 88.3, 89.8, 69.5	80.4 ± 11 (14)
	0.40	99.3, 95.5, 99.0, 94.8, 96.5	97.0 ± 2.0 (2.1)
	10	101, 94.1, 105, 98.9, 104	101 ± 4.4 (4.3)
Cotton meal	0.02	98.5, 108, 102, 103, 102	102 ± 3.3 (3.2)
	0.20	87.5, 86.0, 84.5, 87.5, 84.5	86.0 ± 1.5 (1.7)
Cotton hulls	0.02	116, 117, 115, 121, 112	116 ± 3.4 (2.9)
	0.20	88.5, 86.0, 85.5, 84.0, 82.0	85.2 ± 2.4 (2.8)
Cotton alkali refined oil	0.02	100, 102, 98.5, 103, 113	103 ± 5.7 (5.5)
	0.20	96.5, 89.0, 91.5, 91.5, 90.5	91.8 ± 2.8 (3.1)

TABLE C.1.1 Recovery Results from Method Validation of Cotton Matrices using the Data-Gathering Analytical Method. Standards were prepared in acetonitrile.			
Matrix	Spiking Level (ppm)	Recoveries Obtained[1] (%)	Mean Recovery ± SD (CV)[1] (%)
DCSA			
Cotton seed	0.005	88, 95, 90, 134, 98	101 ± 19 (19)
	0.01	70, 84, 102, 96, 98	89.8 ± 13 (15)
	0.02	99.5, 82.0, 97.0, 97.0, 94.5	94.0 ± 6.9 (7.4)
	0.20	94.5, 99.5, 105, 95.5, 101	98.9 ± 4.0 (4.1)
	10	80.7, 92.1, 95.1, 93.1, 96.9	91.6 ± 6.4 (6.9)
Cotton gin byproducts	0.04	79.0, 87.3, 75.5, 75.3, 80.5	79.5 ± 4.9 (6.1)
	0.40	90.5, 83.5, 92.8, 87.0, 91.0	89.0 ± 3.7 (4.2)
	10	110, 101, 91.8, 109, 98.8	102 ± 7.6 (7.4)
Cotton meal	0.02	101, 95.5, 100, 89.5, 97.5	96.5 ± 4.4 (4.5)
	0.20	82.0, 83.5, 85.5, 91.0, 89.0	86.2 ± 3.8 (4.4)
Cotton hulls	0.02	109, 102, 93.5, 97.0, 96.0	100 ± 6.1 (6.2)
	0.20	95.5, 102, 91.5, 100, 99.0	97.4 ± 3.9 (4.0)
Cotton alkali refined oil	0.02	102, 93.0, 89.5, 92.5, 100	95.2 ± 5.1 (5.3)
	0.20	103, 106, 100, 104, 105	103 ± 2.1 (2.0)

[1] As calculated by the reviewer.

TABLE C.1.2 Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Dicamba and Dicamba Metabolites in Cotton Matrices.	
Analyte(s)	Dicamba, 5-OH dicamba, DCSA and DCGA
Equipment ID	Shimadzu Prominence HPLC equipped with a Betasil Phenyl HPLC column (50 mm x 3 mm x 3 µm). Quantitation of residues is performed using an Applied Biosystems API 5500 MS/MS using turbo ion spray ionization operated in the negative-ion multiple reaction monitoring (MRM) mode.
Limit of quantitation (LOQ)	The LOQs, determined as the LLMV, were 0.02 ppm for each analyte in cotton seed, meal, hulls, and oil; and 0.04 ppm in gin by products. Calculated LOQs were determined for cotton seed based on replicate determinations at the LLMV. The LOQs were 0.0266 ppm for dicamba, 0.0171 ppm for 5-OH dicamba, 0.0105 ppm for DCSA, and 0.0121 ppm DCGA.
Limit of detection (LOD)	The LODs, defined as the $t_{0.99}$ confidence interval times the standard deviation of the fortified replicates at the LLMV, were determined for cotton seed. The LODs were 0.0089 ppm for dicamba, 0.0057 ppm for 5-OH dicamba, 0.0035 ppm for DCSA and 0.0041 ppm for DCGA.
Accuracy/Precision	Percent recoveries and coefficients of variance (CVs) indicate acceptable accuracy/precision at 0.02, 0.20, and 10 ppm for dicamba and its metabolites in seed; at 0.02 and 0.20 ppm in meal, hulls, and oil; and at 0.04, 0.40, and 10 ppm for gin byproducts. Average recovery ranges (and CVs) were 86.7-113% (1.22-14.6) for dicamba, 87.1-111% (2.90-16.1) for 5-OH dicamba, 79.5-103% (2.01-7.40) for DCSA, and 80.4-116 (1.74-14.0) for DCGA. See Table C.1.1 above.
Reliability of the Method/ [ILV]	An independent laboratory method validation [ILV] was not conducted.
Linearity	The method/detector response was linear (coefficient of determination, $r^2 = \geq 0.99$) within the range of 0.005 - 0.50 µg/mL.

TABLE C.1.2	Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Dicamba and Dicamba Metabolites in Cotton Matrices.
Specificity	The control chromatograms generally have no peaks above the chromatographic background and the spiked sample chromatograms contain only the analyte peak of interest. In cotton gin byproducts, however, DCGA and 5-OH dicamba each had matrix interference for at least one lot that exceeded 30% of the LLMV. The study notes that chromatographic interference may be a problem in some matrices. Gradient conditions and use of MS with MRM analysis greatly reduces the interferences for these samples. The study also notes that there are no interferences from 2,4-dichlorophenoxyacetic acid or 2,4-dichlorophenylacetic acid, which have the same mass transitions as dicamba and DCSA, respectively. Interferences from other pesticides are unknown, however, none are expected due to the high level of specificity of the LC/MS/MS analysis. Peaks were well defined and symmetrical. There appeared to be no carryover to the following chromatograms.

Table C.1.3. Extraction Efficiency of Method AG-ME-1381-01 As Compared to the Metabolism Study Extraction Method

Matrix	Analyte	Extraction Method	Radioactive Residues (ppm) [Average]	Extraction Efficiency[1] (%)
Seed	Dicamba	Metabolism study	0.0102	106
		Method AG-ME-1381-01	0.0098, 0.0119 [0.0108]	
	DCGA	Metabolism study	0.0224	101
		Method AG-ME-1381-01	0.0227, 0.0227 [0.0227]	
	DCSA	Metabolism study	0.0532	121
		Method AG-ME-1381-01	0.0634, 0.0658 [0.0646]	
Gin Byproduct	Dicamba	Metabolism study	2.83	83
		Method AG-ME-1381-01	2.31, 2.37 [2.34]	
	DCGA	Metabolism study	4.63	79
		Method AG-ME-1381-01	3.82, 3.50 [3.66]	
	DCSA	Metabolism study	16.1	87
		Method AG-ME-1381-01	13.99, 14.09 [14.04]	

[1] Extraction efficiency = (residues determined by residue method ÷ residues determined in metabolism study)*100.

C.2. Enforcement Method

A gas chromatography method with electron capture detection (GC/ECD) is the current enforcement method for residues of dicamba in/on plant commodities (Method AM-0691B-0297-4).

C.3. Independent Laboratory Validation

An independent laboratory validation (ILV) study was not included as a part of this submission; however, no additional information is required because ILV is not generally required for data collection methods.

D. CONCLUSION

Adequate method validation data have been submitted for the analytical method AG-ME-1381-01 for the determination of residues of dicamba, 5-OH dicamba, DCSA and DCGA in cotton commodities. The method has been adequately validated with accuracy and precision over the range of 0.02 to 10 ppm for cotton seed; 0.02 to 0.20 ppm for meal, hulls, and oil, and 0.04 to 10 ppm for cotton gin byproducts. Mean recoveries were within acceptable range of 70% to 120% with the coefficient of variation less than 20% for each matrix tested. The data are sufficiently representative of the expected residue levels in cotton seed, gin byproducts, meal, hulls, and oil. Acceptable radiovalidation data were submitted.

No confirmatory analysis procedures were included for the method, and no interference study was submitted; however, because the method has not been proposed for enforcement, confirmatory analysis procedures are not required.

An ILV was not submitted and is not required in support of a data collection method.

E. REFERENCES

DP No.: D317699
Subject: Dicamba. Residue Chemistry Considerations for the Reregistration Eligibility Decision (RED) Document. Summary of Analytical Chemistry and Residue Data.
From: C. L. Olinger
To: K. Tyler
Date: 12/20/2005
MRIDs: None

F. DOCUMENT TRACKING

Petition Number(s): 2F8067
DP Barcode(s): 408384
PC Code: 029801, 029802, 029806, 128931, 128944, & 129043

Template Version September 2003

Primary Evaluator		Date: 11/15/2012
	Versar, Inc.	
Approved by	Peter Savoia	Date: 04/22/2013
	Peter Savoia, Chemist, OCSPP-HED-RAB V/VII	

This DER was originally prepared under contract by Versar, Inc. (6850 Versar Center, Springfield, VA 22151; submitted (11/15/2012). The DER has been reviewed by HED and revised to reflect current Office of Pesticide Programs (OPP) policies.

STUDY REPORT:

48728703 Maher, D.; Foster, J. (2011) Magnitude of Dicamba Residues in Cotton Raw Agricultural and Processed Commodities following Applications of Dicamba-Based Formulations to MON 88701. Project Number: REG/10/062/OCR, MSL0024072. Unpublished study prepared by Monsanto Company, Carringers, Inc. and GLP Technologies. 603 p.

EXECUTIVE SUMMARY:

Monsanto has submitted field trial data for dicamba on dicamba-tolerant cotton, identified as Event MON 88701 (formerly GH_S26695). Event MON 88701 cotton expresses a modified dicamba mono-oxygenase (DMO) gene derived from *Stenotrophomonas maltophilia* and the bialaphos resistance (BAR) gene isolated from *Streptomyces hygroscopicus*. The expression of DMO confers dicamba tolerance and the expression of BAR confers glufosinate tolerance to Event MON 88701 cotton. Thirteen field trials were conducted in the United States during the 2010 growing season in the North American Free Trade Agreement (NAFTA) Growing Zones 2 (GA and SC; 2 trials), 4 (AR, LA, and MO; 3 trials), 6 (TX; 1 trial), 8 (OK and TX; 5 trials), and 10 (CA; 2 trials).

Each trial consisted of one untreated and three to four treated plots. Each treated plot followed one of three treatment types: three broadcast applications applied at pre-emergence, 6-leaf, and 1st white flower + 15 days [TRT 2]; three broadcast applications applied at pre-emergence, first open boll, and 7-days prior to harvest [TRT 3]; and four foliar broadcast applications applied at 6-leaf, 1st white flower + 15 days, first open boll, and 7 days prior to harvest [TRTs 4 and 5]. Applications to plots 2, 3, and 4 were made using a 4 lb ae/gal water soluble concentrate (SL) formulation of dicamba diglycolamine salt (Clarity Herbicide; MON 54140); plot 5 applications were made using a 5 lb ae/gal SL formulation of dicamba monoethanolamine salt (MON 11968) to provide bridging data. The total application rate for all treatment types was 2.0-2.1 lb ae/A (2.2-2.4 kg ae/ha). For TRT 2, application 1 was applied at 0.98-1.0 lb ae/A (1.1 kg ae/ha) and applications 2 and 3 were applied at 0.49-0.51 lb ae/A/application (0.55-0.57 kg ae/ha/application). Retreatment intervals (RTIs) were 24-49 days between the first and second applications and 36-49 days between the second and third applications. For TRT 3, application 1 was applied at 0.99-1.0 lb ae/A (1.1 kg ae/ha) and applications 2 and 3 were applied at 0.49-0.52 lb ae/A/application (0.55-0.58 kg ae/ha/application), for a total seasonal rate of 2.0 lb ae/A (2.2 kg ae/ha). RTIs were 97-178 days between the first and second applications and 5-63 days between the second and third applications. For TRTs 4 and 5, applications were applied at 0.49-

0.52 lb ae/A/application (0.55-0.58 kg ae/ha/application). RTIs were 36-49 days between the first and second applications, 32-88 days between the second and third applications, and 5-63 days between the third and fourth applications. A summary of the use patterns is provided in the table below.

TRT	Number of Sites	EP	Growth Stages/ Target Application Rates lb ae/A (kg ae/ha)				
			Pre-emergence	6 Leaf	1st White Flower + 15 days	First Open Boll	7 Days Prior to Harvest
1	13	--	--	--	--	--	--
2	13	4 lb ae/gal SL	1.0 (1.1)	0.50 (0.56)	0.50 (0.56)	--	--
3	13	4 lb ae/gal SL	1.0 (1.1)	--	--	0.50 (0.56)	0.50 (0.56)
4	13	4 lb ae/gal SL	--	0.50 (0.56)	0.50 (0.56)	0.50 (0.56)	0.50 (0.56)
5	4	5 lb ae/gal SL	--	0.50 (0.56)	0.50 (0.56)	0.50 (0.56)	0.50 (0.56)

Applications for all treatments were made using ground equipment (backpack, hand-held and tractor-mounted sprayers) in spray volumes of 18.8-21.6 gal/A (176-202 l/ha) and included an nonionic surfactant (NIS) and ammonium sulfate (AMS) at all trial sites.

Undelinted cotton seed was collected mechanically with a cotton picker (n=3) or a cotton stripper (n=3) or by hand (n=7) at normal harvest: 49-105-day preharvest interval (PHI) for TRT 2 and 6-8-day PHI for TRTs 3-5. Additional samples of cotton seed were collected from two trials at PHIs of 43/99, 49/105, 56/112, and 63/119 days for TRT 2 and 1, 7, 14, and 21 days for TRT 4 to assess residue decline. Cotton gin byproduct samples were collected from three sites using a cotton stripper at normal harvest (82-84-day PHI for TRT 2; 6-7-day PHI for TRT 4).

Samples were analyzed for residues of dicamba and metabolites 5-hydroxydicamba (5-OH dicamba), 3,6-dichlorosalicylic acid (DCSA), and 3,6-dichlorogentisic acid (DCGA) using a high performance liquid chromatography method with tandem mass spectrometry detection (LC/MS/MS). The limit of quantitation (LOQ, equivalent to the lowest level of method validation; LLMV) was 0.02 ppm for all analytes in undelinted cotton seed and 0.04 ppm for all analytes in cotton gin byproducts. The method was adequate for data validation based on acceptable method and concurrent recovery data. The fortification levels used in concurrent method recovery were adequate to bracket expected residue levels. Concurrent recoveries were corrected for apparent residues in controls; residues in treated samples were not corrected for residues in controls. We note that the analytical method does not specify conversion of metabolite residues to parent equivalents; therefore, quantifiable residues of 5-OH dicamba, DCSA, and DCGA were converted to parent equivalents by the study reviewer using a molecular weight conversion factor of 0.933, 1.068, and 0.991, respectively.

Dicamba, 5-OH dicamba, and DCSA undelinted cotton seed samples were stored for 13-146 days (0.4-4.8 months) from collection to analysis; DCGA undelinted cotton seed samples were stored for 13-285 days (0.4-9.4 months). Gin byproduct samples for all analytes were stored from 17-27 days (0.6-0.9 months) from collection to analysis. All samples were analyzed within 0 to 2 days of extraction. Samples were stored below freezing at the field sites and at -20 °C at the analytical laboratory prior to extraction. A storage stability study for dicamba, DCGA, and

DCSA on cotton was submitted in conjunction with the field trial study (refer to the 860.1380 der for MRID 48728704). The stability of 5-OH dicamba was not determined concurrently because it was not found in undelinted cotton seed. These data are acceptable to support the storage conditions and durations of the undelinted cotton seed samples from the submitted field trials. Storage stability data for cotton gin byproduct was not provided; however, storage stability data is not required as the samples were analyzed within 30 days of harvest.

Following three broadcast applications of the 4 lb ae/gal SL formulation of dicamba (formulated as the diglycolamine salt), which were applied at pre-emergence, the 6-leaf stage, and first white flower + 15 days for a total rate of 2.0 lb ae/A (2.2 kg ae/ha) (**TRT 2**), combined residues (and per trial averages) of dicamba, 5-OH dicamba, DCGA, and DCSA (expressed in parent equivalents) were <0.08-<0.39 (<0.08-<0.39) ppm in/on undelinted cotton seed collected at PHIs of 49-105 days and <0.67-<2.34 (<0.73-<2.33) ppm in/on gin byproducts collected at PHIs of 82-84 days.

Following three broadcast applications of the 4 lb ae/gal SL formulation of dicamba (formulated as the diglycolamine salt), which were applied at pre-emergence, the first open boll stage, and 7 days prior to harvest for a total rate of 2.0 lb ae/A (2.2 kg ae/ha) (**TRT 3**), combined residues (and per trial averages) of dicamba, 5-OH dicamba, DCGA, and DCSA (expressed in parent equivalents) were <0.12-<2.24 (<0.13-<1.66) ppm in/on undelinted cotton seed collected at a 6-8-day PHI.

Following four foliar broadcast applications of the 4 lb ae/gal SL formulation of dicamba (formulated as the diglycolamine salt), which were applied at the 6-leaf stage, the first white flower + 15 days, the first open boll, and 7 days prior to harvest for a total rate of 2.0-2.1 lb ae/A (2.2-2.4 kg ae/ha) (**TRT 4**), combined residues (and per trial averages) of dicamba, 5-OH dicamba, DCGA, and DCSA (expressed in parent equivalents) were <0.15-<2.02 (<0.21-<1.86) ppm in/on undelinted cotton seed collected at a 6-8-day PHI and <5.50-34.6 (<5.51-<33.7) ppm in/on gin byproducts collected at a 6-7-day PHI. Corresponding combined residues (and per trial averages) in side-by-side trials treated with the 5 lb ae/gal SL formulation of dicamba formulated as the monoethanolamine salt (**TRT 5**) were <0.24-<1.00 (<0.29-<0.82) ppm in/on undelinted cotton seed. Average combined residues in the diglycolamine salt formulation trials were slightly higher (approximately 1.5x) than the corresponding residues in the monoethanolamine salt formulation trials.

Treatment plots 3 and 4 showed the highest average combined residues (0.76 ppm), followed closely by treatment plot 5 (average of 0.52 ppm). Treatment plot 2 had the lowest average combined residues (average of 0.12 ppm). Lower residue levels obtained from treatment plot 2 are consistent with the earlier application timings at these plots.

For the TRT 2 residue decline samples, average residues of dicamba, 5-OH dicamba, DCGA, and DCSA in/on undelinted cotton seed were at or <LOQ in both studies; therefore, residue decline could not be assessed. For the TRT 4 residue decline samples, average residues of dicamba decreased in/on undelinted cotton seed with increasing PHI. Average residues of

DCGA and DCSA increased with increasing PHI in the SC1 trial, and remained relatively constant in the TX1 trial. Residues of 5-OH dicamba were <LOQ in both studies; therefore, residue decline could not be assessed.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the field trial residue data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document D408384.

COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance, and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Dicamba (3,6-dichloro-2-methoxybenzoic acid) is a selective benzoic acid herbicide registered for the control of weeds prior to their emergence. The Dicamba Reregistration Eligibility Decision (RED) was issued December 2005. The chemical structure and nomenclature of dicamba and its metabolites 5-OH dicamba, DSCA, and DCGA are presented in Table A.1. The physicochemical properties of the technical grade of dicamba acid are presented in Table A.2.

TABLE A.1. Test Compound Nomenclature.

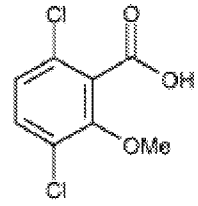
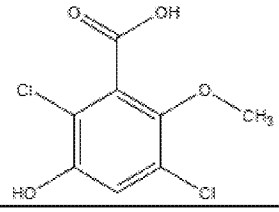
Compound	
Common name	Dicamba
Company experimental name	MON 11900
IUPAC name	3,6-dichloro-o-anisic acid or 3,6-dichloro-2-methoxybenzoic acid
CAS name	3,6-dichloro-2-methoxybenzoic acid
CAS registry number	1918-00-9 (dicamba acid), 104040-79-1 (diglycolamine salt), or 53404-28-7 (monoethanolamine salt)
End-use product	Clarity® Herbicide: SL formulation containing 4 lb ae/gal MON 11968: SL formulation containing 5 lb ae/gal
Compound	

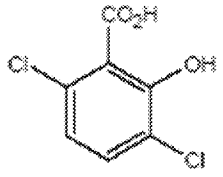
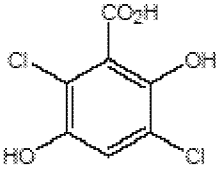
TABLE A.1. Test Compound Nomenclature.	
Common name	5-Hydroxy-dicamba
Company experimental name	5-OH dicamba
IUPAC/CAS name	2,5-dichloro-3-hydroxy-6-methoxybenzoic acid
CAS registry number	7600-50-2
Compound	
Common name	DCSA; 3,6-dichlorosalicylic acid
Company experimental name	MON 52708
IUPAC/CAS name	3,6-dichloro-2-hydroxybenzoic acid
CAS registry number	3401-80-7
Compound	
Common name	DCGA; 3,6-dichlorogentistic acid
Company experimental name	MON 52724
IUPAC/CAS name	2,5-dichloro-3,6-dihydroxybenzoic acid
CAS registry number	18688-01-2

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound: Dicamba.		
Parameter	Value	Reference
Melting point	114-116 EC (PAI) 90-100 EC (87% TGAI)	Residue Chemistry Chapter of the Dicamba RED, DP# 317699, 12/20/05, C. Olinger
pH	2.5-3.0 (87% TGAI)	
Density	1.57 g/mL at 25 EC (87% TGAI)	
Water solubility	0.5 g/100 mL at 25 EC (PAI)	
Solvent solubility	g/100 mL at 25 EC (PAI) dioxane 118.0 ethanol 92.2 isopropyl alcohol 76.0 methylene chloride 26.0 acetone 17.0 toluene 13.0 xylene 7.8 heavy aromatic naphthalene 5.2	
Vapor pressure	3.4 x 10^{-5} mm Hg at 25 EC (PAI)	
Dissociation constant, pK_a	1.97 (PAI)	
Octanol/water partition coefficient, Log(K_{OW})	0.1 (PAI)	
UV/visible absorption spectrum	neutral: 511 (275 nm) acidic (pH 0-1): 1053 (281 nm) basic (pH 13-14): 469 (274 nm)	

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

Thirteen field trials were conducted on cotton in the United States during the 2010 growing season in NAFTA Zones 2 (GA and SC; 2 trials), 4 (AR, LA, and MO; 3 trials), 6 (TX; 1 trial), 8 (OK and TX; 5 trials), and 10 (CA; 2 trials).

Each trial consisted of one untreated and three to four treated plots. The plots were planted with dicamba-tolerant cotton, identified as Event MON 88701 (formerly GH_S26695). Event MON 88701 cotton expresses a modified dicamba mono-oxygenase (DMO) gene derived from *Stenotrophomonas maltophilia* and the bialaphos resistance (BAR) gene isolated from *Streptomyces hygroscopicus*. The expression of DMO confers dicamba tolerance and the expression of BAR confers glufosinate tolerance to Event MON 88701 cotton. Each treated plot followed one of three treatment types: three broadcast applications applied at pre-emergence, 6-leaf, and 1st white flower + 15 days [TRT 2]; three broadcast applications applied at pre-emergence, first open boll, and 7-days prior to harvest [TRT 3]; and four foliar broadcast applications applied at 6-leaf, 1st white flower + 15 days, first open boll, and 7 days prior to harvest [TRTs 4 and 5]. Applications to plots 2, 3, and 4 were made using a 4 lb ae/gal SL formulation of dicamba diglycolamine salt (Clarity Herbicide; MON 54140); plot 5 applications were made using a 5 lb ae/gal SL formulation of dicamba monoethanolamine salt (MON 11968) to provide bridging data. The total application rate for all treatment types was 2.0-2.1 lb ae/A (2.2-2.4 kg ae/ha). For TRT 2, application 1 was applied at 0.98-1.0 lb ae/A (1.1 kg ae/ha) and applications 2 and 3 were applied at 0.49-0.51 lb ae/A/application (0.55-0.57 kg ae/ha/application). RTIs were 24-49 days between the first and second applications and 36-49 days between the second and third applications. For TRT 3, application 1 was applied at 0.99-1.0 lb ae/A (1.1 kg ae/ha) and applications 2 and 3 were applied at 0.49-0.52 lb ae/A/application (0.55-0.58 kg ae/ha/application). RTIs were 97-178 days between the first and second applications and 5-63 days between the second and third applications. For TRTs 4 and 5, applications were applied at 0.49-0.52 lb ae/A/application (0.55-0.58 kg ae/ha/application). RTIs were 36-49 days between the first and second applications, 32-88 days between the second and third applications, and 5-63 days between the third and fourth applications. A summary of the use patterns is provided in the table below.

TRT	Number of Sites	EP	Growth Stages/ Target Application Rates lb ae/A (kg ae/ha)				
			Pre-emergence	6 Leaf	1st White Flower + 15 days	First Open Boll	7 Days Prior to Harvest
1	13	--	--	--	--	--	--
2	13	4 lb ae/gal SL	1.0 (1.1)	0.50 (0.56)	0.50 (0.56)	--	--
3	13	4 lb ae/gal SL	1.0 (1.1)	--	--	0.50 (0.56)	0.50 (0.56)
4	13	4 lb ae/gal SL	--	0.50 (0.56)	0.50 (0.56)	0.50 (0.56)	0.50 (0.56)
5	4	5 lb ae/gal SL	--	0.50 (0.56)	0.50 (0.56)	0.50 (0.56)	0.50 (0.56)

Applications for all treatments were made using ground equipment (backpack, hand-held and tractor-mounted sprayers) in spray volumes of 18.8-21.6 gal/A (176-202 l/ha) and included an NIS and an AMS at all trial sites.

Undelinted cotton seed was collected mechanically with a cotton picker (n=3) or a cotton stripper (n=3) or by hand (n=7) at normal harvest: 49-105-day preharvest interval (PHI) for TRT 2 and 6-8-day PHI for TRTs 3-5. Additional samples of cotton seed were collected from two trials conducted in SC and TX at PHIs of 43/99, 49/105, 56/112, and 63/119 days for TRT 2 and 1, 7, 14, and 21 days for TRT 4 to assess residue decline; decline sampling times corresponded to 1 day following the 7-day PHI application, normal harvest, 7±1 days following normal harvest, and 14±1 days following normal harvest. Cotton gin byproduct samples were collected from three sites (in TX and OK) using a cotton stripper at normal harvest (82-84-day PHI for TRT 2; 6-7-day PHI for TRT 4).

For each field trial, the petitioner presented the average minimum and maximum monthly temperatures and the total monthly rainfall for the study period, as well as the historical averages. Irrigation was used to supplement rainfall as needed. The petitioner reported that no unusual weather events occurred that negatively affected the residue trials; however, precipitation was greater than normal at the CA2, TX1, and TX4 sites and less than normal at the LA1, OK1, and OK2 sites. These conditions did not cause adverse crop growth and development, except at CA2 where cool temperatures and excess moisture in October and November kept the cotton from maturing at a normal rate.

Trial conditions are presented in Table B.1.1. The study use pattern is presented in Table B.1.2, and the crop varieties grown are identified in Table C.3.

TABLE B.1.1 Trial Site Conditions.

Trial Identification: City, County, State; Year (Trial No.)	Soil characteristics			
	Type	%OM[1]	pH[1]	CEC[1] meq/100 g
Proctor, Crittenden, AR; 2010 (AR1)	Clay	1.1	6.3	17.4
Porterville, Tulare, CA; 2010 (CA1)	Sandy loam	0.5	8.2	Not reported
Visalia, Tulare, CA; 2010 (CA2)	Sandy loam	1.11	7.2	5.6
Chula, Tift, GA; 2010 (GA1)	Loamy sand	Not reported		
Cheneyville, Rapides, LA; 2010 (LA1)	Silt loam	0.88	8.07	13.77
Fisk, Butler, MO; 2010 (MO1)	Silt loam	1.4	5.2	9.0
Hinton, Caddo, OK; 2010 (OK1)	Fine sandy loam	1.6	6.6	12.5
Dill City, Washita, OK; 2010 (OK2)	Loamy sand	0.6	7.4	6.0
Elko, Barnwell, SC; 2010 (SC1)	Loamy sand	1.0	6.1	3.6
Raymondville, Willacy, TX; 2010 (TX1)	Clay loam	1.1	8.2	26.7
Levelland, Hockley, TX; 2010 (TX2)	Sandy loam	0.59	7.9	10.35
Wolfforth, Lubbock, TX; 2010 (TX3)	Sandy clay loam	0.6	7.6	10.1
Uvalde, Uvalde, TX; 2010 (TX4)	Clay loam	2.2	7.9	25.4

[1] These parameters are optional except in cases where their value affects the use pattern for the chemical.

TABLE B.1.2. Study Use Pattern.

Location City, County, State;Year (Trial ID)	EP[1]	TRT	Application					Tank Mix/ Adjuvants[3]	Harvest Procedures
			Method; Timing	Volume gal/A (l/ha)	Rate lb ae/A (kg ae/ha)	RTI[2] (days)	Total Rate lb ae/A (kg ae/ha)		
Proctor, Crittenden, AR; 2010 (AR1)	4 lb ae/gal SL	2	1. Soil broadcast; Preemergence	20.1 (188)	1.0 (1.1)	--	2.0 (2.2)	NIS + AMS	Mechanical picker
			2. Foliar broadcast; 6-leaf	20.0 (187)	0.50 (0.56)	29			
			3. Foliar broadcast; 1st WF + 15 days	20.1 (188)	0.50 (0.56)	38			
	4 lb ae/gal SL	3	1. Soil broadcast; Preemergence	20.1 (188)	1.0 (1.1)	--	2.0 (2.2)	NIS + AMS	Mechanical picker
			2. Foliar broadcast; 1st open boll	20.1 (188)	0.50 (0.56)	99			
			3. Foliar broadcast; 7 days prior to harvest	20.1 (188)	0.50 (0.56)	31			
	4 lb ae/gal SL	4	1. Foliar broadcast; 6-leaf	20.1 (188)	0.50 (0.56)	--	2.0 (2.2)	NIS + AMS	Mechanical picker
			2. Foliar broadcast; 1st WF + 15 days	20.2 (189)	0.50 (0.56)	38			
			3. Foliar broadcast; 1st open boll	20.1 (188)	0.50 (0.56)	32			
			4. Foliar broadcast; 7 days prior to harvest	20.1 (188)	0.50 (0.56)	31			
	5 lb ae/gal SL	5	1. Foliar broadcast; 6-leaf	20.0 (187)	0.50 (0.56)	--	2.0 (2.2)	NIS + AMS	Mechanical picker
			2. Foliar broadcast; 1st WF + 15 days	20.2 (189)	0.50 (0.56)	38			
			3. Foliar broadcast; 1st open boll	20.2 (189)	0.50 (0.56)	32			
			4. Foliar broadcast; 7 days prior to harvest	20.1 (188)	0.50 (0.56)	31			
Porterville, Tulare, CA; 2010 (CA1)	4 lb ae/gal SL	2	1. Soil broadcast; Preemergence	20.5 (192)	1.0 (1.1)	--	2.0 (2.2)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 6-leaf	20.3 (190)	0.50 (0.56)	48			
			3. Foliar broadcast; 1st WF + 15 days	20.1 (188)	0.51 (0.57)	43			
	4 lb ae/gal SL	3	1. Soil broadcast; Preemergence	20.3 (190)	1.0 (1.1)	--	2.0 (2.2)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 1st open boll	20.2 (189)	0.51 (0.57)	136			
			3. Foliar broadcast; 7 days prior to harvest	20.4 (191)	0.51 (0.57)	28			
	4 lb ae/gal SL	4	1. Foliar broadcast; 6-leaf	20.2 (189)	0.50 (0.56)	--	2.0 (2.2)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 1st WF + 15 days	20.3 (190)	0.52 (0.58)	43			
			3. Foliar broadcast; 1st open boll	20.2 (189)	0.51 (0.57)	45			
			4. Foliar broadcast; 7 days prior to harvest	20.2 (189)	0.51 (0.57)	28			

TABLE B.1.2. Study Use Pattern.

Location City, County, State;Year (Trial ID)	EP[1]	TRT	Application					Tank Mix/ Adjuvants[3]	Harvest Procedures
			Method; Timing	Volume gal/A (l/ha)	Rate lb ae/A (kg ae/ha)	RTI[2] (days)	Total Rate lb ae/A (kg ae/ha)		
Porterville, Tulare, CA; 2010 (CA1) (continued)	5 lb ae/gal SL	5	1. Foliar broadcast; 6-leaf	20.2 (189)	0.50 (0.56)	--	2.0 (2.2)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 1st WF + 15 days	20.2 (189)	0.52 (0.58)	43			
			3. Foliar broadcast; 1st open boll	20.1 (188)	0.50 (0.56)	45			
			4. Foliar broadcast; 7 days prior to harvest	20.0 (187)	0.50 (0.56)	28			
Visalia, Tulare, CA; 2010 (CA2)	4 lb ae/gal SL	2	1. Soil broadcast; Preemergence	20.5 (192)	1.0 (1.1)	--	2.0 (2.2)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 6-leaf	20.1 (188)	0.50 (0.56)	41			
			3. Foliar broadcast; 1st WF + 15 days	20.7 (194)	0.51 (0.57)	49			
	4 lb ae/gal SL	3	1. Soil broadcast; Preemergence	20.4 (191)	1.0 (1.1)	--	2.0 (2.2)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 1st open boll	20.3 (190)	0.51 (0.57)	178			
			3. Foliar broadcast; 7 days prior to harvest	20.5 (192)	0.50 (0.56)	7			
	4 lb ae/gal SL	4	1. Foliar broadcast; 6-leaf	20.6 (193)	0.51 (0.57)	--	2.0 (2.2)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 1st WF + 15 days	20.3 (190)	0.50 (0.56)	49			
			3. Foliar broadcast; 1st open boll	20.4 (191)	0.51 (0.57)	88			
			4. Foliar broadcast; 7 days prior to harvest	20.8 (195)	0.51 (0.57)	7			
Chula, Tift, GA; 2010 (GA1)	4 lb ae/gal SL	2	1. Soil broadcast; Preemergence	20.5 (192)	0.99 (1.1)	--	2.0 (2.2)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 6-leaf	20.5 (192)	0.50 (0.56)	26			
			3. Foliar broadcast; 1st WF + 15 days	20.2 (189)	0.51 (0.57)	43			
	4 lb ae/gal SL	3	1. Soil broadcast; Preemergence	20.6 (193)	1.0 (1.1)	--	2.0 (2.2)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 1st open boll	20.4 (191)	0.52 (0.58)	104			
			3. Foliar broadcast; 7 days prior to harvest	20.4 (191)	0.50 (0.56)	42			
	4 lb ae/gal SL	4	1. Foliar broadcast; 6-leaf	20.9 (195)	0.51 (0.57)	--	2.0 (2.2)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 1st WF + 15 days	20.0 (187)	0.50 (0.56)	43			
			3. Foliar broadcast; 1st open boll	19.8 (185)	0.51 (0.57)	35			
			4. Foliar broadcast; 7 days prior to harvest	20.4 (191)	0.50 (0.56)	42			

TABLE B.1.2. Study Use Pattern.

Location City, County, State;Year (Trial ID)	EP[1]	TRT	Application					Tank Mix/ Adjuvants[3]	Harvest Procedures
			Method; Timing	Volume gal/A (l/ha)	Rate lb ae/A (kg ae/ha)	RTI[2] (days)	Total Rate lb ae/A (kg ae/ha)		
Chula, Tift, GA; 2010 (GA1) (continued)	5 lb ae/gal SL	5	1. Foliar broadcast; 6-leaf	20.9 (195)	0.51 (0.57)	--	2.0 (2.2)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 1st WF + 15 days	20.0 (187)	0.50 (0.56)	43			
			3. Foliar broadcast; 1st open boll	19.5 (182)	0.50 (0.56)	35			
			4. Foliar broadcast; 7 days prior to harvest	20.2 (189)	0.50 (0.56)	42			
Cheneyville, Rapides, LA; 2010 (LA1)	4 lb ae/gal SL	2	1. Soil broadcast; Preemergence	20.5 (192)	1.0 (1.1)	--	2.0 (2.2)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 6-leaf	19.0 (178)	0.51 (0.57)	27			
			3. Foliar broadcast; 1st WF + 15 days	19.2 (180)	0.51 (0.57)	37			
	4 lb ae/gal SL	3	1. Soil broadcast; Preemergence	20.8 (195)	1.0 (1.1)	--	2.0 (2.2)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 1st open boll	20.1 (188)	0.50 (0.56)	97			
			3. Foliar broadcast; 7 days prior to harvest	21.6 (202)	0.52 (0.58)	33			
	4 lb ae/gal SL	4	1. Foliar broadcast; 6-leaf	19.2 (180)	0.52 (0.58)	--	2.0 (2.2)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 1st WF + 15 days	19.4 (181)	0.51 (0.57)	37			
			3. Foliar broadcast; 1st open boll	20.5 (192)	0.51 (0.57)	33			
			4. Foliar broadcast; 7 days prior to harvest	21.2 (198)	0.51 (0.57)	33			
Fisk, Butler, MO; 2010 (MO1)	4 lb ae/gal SL	2	1. Soil broadcast; Preemergence	20.0 (187)	1.0 (1.1)	--	2.0 (2.2)	NIS + AMS	Mechanical picker
			2. Foliar broadcast; 6-leaf	20.0 (187)	0.50 (0.56)	29			
			3. Foliar broadcast; 1st WF + 15 days	20.0 (187)	0.50 (0.56)	42			
	4 lb ae/gal SL	3	1. Soil broadcast; Preemergence	20.0 (187)	1.0 (1.1)	--	2.0 (2.2)	NIS + AMS	Mechanical picker
			2. Foliar broadcast; 1st open boll	20.0 (187)	0.50 (0.56)	113			
			3. Foliar broadcast; 7 days prior to harvest	20.0 (187)	0.50 (0.56)	30			
	4 lb ae/gal SL	4	1. Foliar broadcast; 6-leaf	20.3 (190)	0.51 (0.57)	--	2.0 (2.2)	NIS + AMS	Mechanical picker
			2. Foliar broadcast; 1st WF + 15 days	20.1 (188)	0.50 (0.56)	42			
			3. Foliar broadcast; 1st open boll	20.0 (187)	0.50 (0.56)	42			
			4. Foliar broadcast; 7 days prior to harvest	20.0 (187)	0.50 (0.56)	30			

TABLE B.1.2. Study Use Pattern.

Location City, County, State;Year (Trial ID)	EP[1]	TRT	Application					Tank Mix/ Adjuvants[3]	Harvest Procedures
			Method; Timing	Volume gal/A (l/ha)	Rate lb ae/A (kg ae/ha)	RTI[2] (days)	Total Rate lb ae/A (kg ae/ha)		
Hinton, Caddo, OK; 2010 (OK1)	4 lb ae/gal SL	2	1. Soil broadcast; Preemergence	18.8 (176)	0.99 (1.1)	--	2.0 (2.2)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 6-leaf	20.3 (190)	0.51 (0.57)	24			
			3. Foliar broadcast; 1st WF + 15 days	20.1 (188)	0.50 (0.56)	49			
	4 lb ae/gal SL	3	1. Soil broadcast; Preemergence	19.0 (178)	1.0 (1.1)	--	2.0 (2.2)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 1st open boll	19.8 (185)	0.50 (0.56)	107			
			3. Foliar broadcast; 7 days prior to harvest	20.6 (193)	0.51 (0.57)	27			
	4 lb ae/gal SL	4	1. Foliar broadcast; 6-leaf	20.3 (190)	0.51 (0.57)	--	2.0 (2.2)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 1st WF + 15 days	19.9 (186)	0.49 (0.55)	49			
			3. Foliar broadcast; 1st open boll	20.2 (189)	0.51 (0.57)	34			
			4. Foliar broadcast; 7 days prior to harvest	20.6 (193)	0.51 (0.57)	27			
	5 lb ae/gal SL	5	1. Foliar broadcast; 6-leaf	19.8 (185)	0.50 (0.56)	--	2.0 (2.2)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 1st WF + 15 days	19.8 (185)	0.49 (0.55)	49			
			3. Foliar broadcast; 1st open boll	19.7 (184)	0.50 (0.56)	34			
			4. Foliar broadcast; 7 days prior to harvest	20.3 (190)	0.50 (0.56)	27			
Dill City, Washita, OK; 2010 (OK2)	4 lb ae/gal SL	2	1. Soil broadcast; Preemergence	19.0 (178)	0.98 (1.1)	--	2.0 (2.2)	NIS + AMS	Mechanical stripper
			2. Foliar broadcast; 6-leaf	20.1 (188)	0.50 (0.56)	28			
			3. Foliar broadcast; 1st WF + 15 days	19.7 (184)	0.49 (0.55)	49			
	4 lb ae/gal SL	3	1. Soil broadcast; Preemergence	19.2 (180)	0.99 (1.1)	--	2.0 (2.2)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 1st open boll	20.3 (190)	0.50 (0.56)	109			
			3. Foliar broadcast; 7 days prior to harvest	20.1 (188)	0.50 (0.56)	43			
	4 lb ae/gal SL	4	1. Foliar broadcast; 6-leaf	20.2 (189)	0.51 (0.57)	--	2.0 (2.2)	NIS + AMS	Mechanical stripper
			2. Foliar broadcast; 1st WF + 15 days	20.1 (188)	0.50 (0.56)	49			
			3. Foliar broadcast; 1st open boll	20.3 (190)	0.50 (0.56)	32			
			4. Foliar broadcast; 7 days prior to harvest	19.7 (184)	0.49 (0.55)	43			

TABLE B.1.2. Study Use Pattern.

Location City, County, State;Year (Trial ID)	EP[1]	TRT	Application					Tank Mix/ Adjuvants[3]	Harvest Procedures
			Method; Timing	Volume gal/A (l/ha)	Rate lb ae/A (kg ae/ha)	RTI[2] (days)	Total Rate lb ae/A (kg ae/ha)		
Elko, Barnwell, SC; 2010 (SC1)	4 lb ae/gal SL	2	1. Soil broadcast; Preemergence	20.3 (190)	1.0 (1.1)	--	2.0 (2.2)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 6-leaf	20.8 (195)	0.50 (0.56)	29			
			3. Foliar broadcast; 1st WF + 15 days	20.6 (193)	0.50 (0.56)	38			
	4 lb ae/gal SL	3	1. Soil broadcast; Preemergence	20.2 (189)	1.0 (1.1)	--	2.0 (2.2)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 1st open boll	20.8 (195)	0.50 (0.56)	102			
			3. Foliar broadcast; 7 days prior to harvest	20.0 (187)	0.50 (0.56)	63			
	4 lb ae/gal SL	4	1. Foliar broadcast; 6-leaf	20.7 (194)	0.50 (0.56)	--	2.0 (2.2)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 1st WF + 15 days	20.6 (193)	0.50 (0.56)	38			
			3. Foliar broadcast; 1st open boll	20.6 (193)	0.50 (0.56)	35			
			4. Foliar broadcast; 7 days prior to harvest	19.9 (186)	0.50 (0.56)	63			
Raymondville, Willacy, TX; 2010 (TX1)	4 lb ae/gal SL	2	1. Soil broadcast; Preemergence	20.3 (190)	1.0 (1.1)	--	2.0 (2.2)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 6-leaf	20.7 (194)	0.52 (0.58)	36			
			3. Foliar broadcast; 1st WF + 15 days	20.9 (195)	0.52 (0.58)	46			
	4 lb ae/gal SL	3	1. Soil broadcast; Preemergence	20.4 (191)	1.0 (1.1)	--	2.0 (2.2)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 1st open boll	20.7 (194)	0.52 (0.58)	119			
			3. Foliar broadcast; 7 days prior to harvest	20.6 (193)	0.52 (0.58)	5			
	4 lb ae/gal SL	4	1. Foliar broadcast; 6-leaf	20.7 (194)	0.52 (0.58)	--	2.1 (2.4)	NIS + AMS	Harvested by hand
			2. Foliar broadcast; 1st WF + 15 days	20.8 (195)	0.52 (0.58)	46			
			3. Foliar broadcast; 1st open boll	20.7 (194)	0.52 (0.58)	37			
			4. Foliar broadcast; 7 days prior to harvest	20.9 (195)	0.52 (0.58)	5			

TABLE B.1.2. Study Use Pattern.

Location City, County, State;Year (Trial ID)	EP[1]	TRT	Application					Tank Mix/ Adjuvants[3]	Harvest Procedures
			Method; Timing	Volume gal/A (l/ha)	Rate lb ae/A (kg ae/ha)	RTI[2] (days)	Total Rate lb ae/A (kg ae/ha)		
Levelland, Hockley, TX; 2010 (TX2)	4 lb ae/gal SL	2	1. Soil broadcast; Preemergence	20.4 (191)	1.0 (1.1)	--	2.0 (2.2)	NIS + AMS	Mechanical stripper
			2. Foliar broadcast; 6-leaf	20.6 (193)	0.51 (0.57)	36			
			3. Foliar broadcast; 1st WF + 15 days	20.1 (188)	0.50 (0.56)	41			
	4 lb ae/gal SL	3	1. Soil broadcast; Preemergence	20.3 (190)	1.0 (1.1)	--	2.0 (2.2)	NIS + AMS	Mechanical stripper
			2. Foliar broadcast; 1st open boll	19.7 (184)	0.49 (0.55)	118			
			3. Foliar broadcast; 7 days prior to harvest	19.8 (185)	0.49 (0.55)	37			
	4 lb ae/gal SL	4	1. Foliar broadcast; 6-leaf	20.0 (187)	0.49 (0.55)	--	2.0 (2.2)	NIS + AMS	Mechanical stripper
			2. Foliar broadcast; 1st WF + 15 days	20.0 (187)	0.49 (0.55)	41			
			3. Foliar broadcast; 1st open boll	20.4 (191)	0.50 (0.56)	41			
			4. Foliar broadcast; 7 days prior to harvest	20.0 (187)	0.49 (0.55)	37			
Wolfforth, Lubbock, TX; 2010 (TX3)	4 lb ae/gal SL	2	1. Soil broadcast; Preemergence	20.1 (188)	1.0 (1.1)	--	2.0 (2.2)	NIS + AMS	Mechanical stripper
			2. Foliar broadcast; 6-leaf	20.7 (194)	0.51 (0.57)	41			
			3. Foliar broadcast; 1st WF + 15 days	19.8 (185)	0.49 (0.55)	36			
	4 lb ae/gal SL	3	1. Soil broadcast; Preemergence	20.2 (189)	1.0 (1.1)	--	2.0 (2.2)	NIS + AMS	Mechanical stripper
			2. Foliar broadcast; 1st open boll	20.0 (187)	0.49 (0.55)	125			
			3. Foliar broadcast; 7 days prior to harvest	19.7 (184)	0.49 (0.55)	30			
	4 lb ae/gal SL	4	1. Foliar broadcast; 6-leaf	20.2 (189)	0.50 (0.56)	--	2.0 (2.2)	NIS + AMS	Mechanical stripper
			2. Foliar broadcast; 1st WF + 15 days	20.1 (188)	0.50 (0.56)	36			
			3. Foliar broadcast; 1st open boll	20.2 (189)	0.50 (0.56)	48			
			4. Foliar broadcast; 7 days prior to harvest	20.1 (188)	0.50 (0.56)	30			

TABLE B.1.2. Study Use Pattern.

Location City, County, State;Year (Trial ID)	EP[1]	TRT	Application					Tank Mix/ Adjuvants[3]	Harvest Procedures
			Method; Timing	Volume gal/A (l/ha)	Rate lb ae/A (kg ae/ha)	RTI[2] (days)	Total Rate lb ae/A (kg ae/ha)		
Uvalde, Uvalde, TX; 2010 (TX4)	4 lb ae/gal SL	2	1. Soil broadcast; Preemergence	20.2 (189)	1.0 (1.1)	--	2.0 (2.2)	NIS + AMS	Mechanical picker
			2. Foliar broadcast; 6-leaf	20.1 (188)	0.50 (0.56)	49			
			3. Foliar broadcast; 1st WF + 15 days	19.9 (186)	0.50 (0.56)	47			
	4 lb ae/gal SL	3	1. Soil broadcast; Preemergence	19.9 (186)	1.0 (1.1)	--	2.0 (2.2)	NIS + AMS	Mechanical picker
			2. Foliar broadcast; 1st open boll	21.5 (201)	0.52 (0.58)	132			
			3. Foliar broadcast; 7 days prior to harvest	19.7 (184)	0.49 (0.55)	29			
	4 lb ae/gal SL	4	1. Foliar broadcast; 6-leaf	20.0 (187)	0.50 (0.56)	--	2.0 (2.2)	NIS + AMS	Mechanical picker
			2. Foliar broadcast; 1st WF + 15 days	19.7 (184)	0.49 (0.55)	47			
			3. Foliar broadcast; 1st open boll	20.5 (192)	0.49 (0.55)	36			
			4. Foliar broadcast; 7 days prior to harvest	20.2 (189)	0.51 (0.57)	29			

[1] EP = End-use Product; Clarity [SL formulation containing 480 g/L dicamba formulated as the diglycolamine salt (4 lb ae/gal)] and MON 11968 [SL formulation containing 600 g/L dicamba formulated as the monoethanolamine salt (5 lb ae/gal).
[2] Retreatment Interval.
[3] NIS = nonionic surfactant. AMS = ammonium sulfate.

TABLE B.1.3. Trial Numbers and Geographical Locations.

NAFTA Growing Zones	Cotton		
	Submitted	Requested[1]	
		Canada	U.S.
1	--	--	--
1A	--	--	--
2	2	--	1/1
3	--	--	--
4	3	--	3/2
5	--	--	--
5A	--	--	--
5B	--	--	--
6	1	--	1/1
7	--	--	--
7A	--	--	--
8	5	--	4/3
9	--	--	--
10	2	--	3/2
11	--	--	--
12	--	--	--
13	--	--	--
14	--	--	--
15	--	--	--
16	--	--	--
17	--	--	--
18	--	--	--
19	--	--	--
20	--	--	--
21	--	--	--
Total	13		12/9

[1] As per OCSPP 860.1500, Tables 1 and 5 for cotton; the second number reflects a 25% reduction in the number of trials allowed for the crop as a representative commodity in support of a crop group/subgroup tolerance or when application results in no quantifiable residues.

B.2. Sample Handling and Preparation

At all test sites, one untreated and duplicate treated cotton seed samples were harvested at normal harvest at a PHI of 49-105 days for TRT 2 and 6-8 days for TRTs 3-5. Two sites (SC1 and TX1) collected additional treated decline samples. The cotton seed samples were harvested by mechanical picker, stripper or by hand and ginned to yield ~1.0 kg of undelinted seed samples (all sites) and ~0.5-1.0 kg of gin byproduct samples (3 sites only). Samples were ginned at the field sites within 3 hours after harvest, except for sites OK2, TX2, and TX3 where the cotton was ginned 1 day after harvest. The treated samples were placed in frozen storage at the field trials within 3.1 hours after sampling. All samples were shipped 0 to 41 days after collection by freezer truck or shipped by FedEx overnight to Monsanto (St. Louis, MO) for residue analysis. Samples were maintained frozen (-20 °C) at the analytical laboratory prior to homogenization and analysis. In preparation for analysis, the cotton gin byproduct samples were initially ground with dry ice in a vertical cutter/mixer. Undelinted cotton seed and gin byproduct samples were further processed by milling in a cryomill, which pulverizes samples into a fine powder at cryogenic temperatures.

B.3. Analytical Methodology

Samples were analyzed for residues of dicamba and the metabolites DCGA, DCSA, and 5-hydoxy dicamba using a LC/MS/MS method from Monsanto Company: Analytical Method AG-ME-1381-01. For a complete description of the LC/MS/MS method, refer to the 860.1340 DER for MRID 48728702. We note that the analytical method does not specify conversion of metabolite residues to parent equivalents; therefore, quantifiable residues of 5-OH dicamba, DCSA, and DCGA were converted to parent equivalents by the study reviewer using a molecular weight conversion factor of 0.933, 1.068, and 0.991, respectively.

Briefly, samples were extracted with acetonitrile:water (40:60, v:v). An aliquot of the extract was hydrolyzed in 1 N HCl at 95 °C in an oven. The hydrolysate was partitioned with ethyl acetate:isooctane (20:80, v:v). Water was added to the organic phase and the sample was concentrated by evaporation until only the aqueous solution remained. The samples were acidified and analyzed using LC/MS/MS.

The LOQ, determined as the LLMV, was 0.02 ppm for each analyte in undelinted cotton seed and 0.04 ppm for each analyte in cotton gin by-products. The 0.020 ppm fortification level was used to statistically determine the LODs and LOQs for the four analytes of the method in undelinted cotton seed. The LODs for the analytes in undelinted cotton seed were 0.0035, 0.0041, 0.0057 and 0.0089 ppm for DCSA, DCGA, 5-hydroxydicamba and dicamba, respectively. The LOQs for the analytes in undelinted cotton seed were 0.0105, 0.0121, 0.0171 and 0.0266 ppm for DCSA, DCGA, 5-hydroxydicamba and dicamba, respectively (reported as analyte found per se). The LODs and LOQs were not statistically determined in cotton gin byproducts.

The analytical method was validated prior to and in conjunction with the analysis of field trial samples.

C. RESULTS AND DISCUSSION

Sample storage conditions and durations are summarized in Table C.2. Dicamba, 5-OH dicamba, and DCSA undelinted cotton seed samples were stored for 13-146 days (0.4-4.8 months) from collection to analysis; DCGA undelinted cotton seed samples were stored for 13-285 days (0.4-9.4 months). Gin byproduct samples for all analytes were stored from 17-27 days (0.6-0.9 months) from collection to analysis. All samples were analyzed within 0 to 2 days of extraction. Samples were stored below freezing at the field sites and at -20 °C at the analytical laboratory prior to extraction. A storage stability study for dicamba, DCGA, and DCSA on cotton was submitted in conjunction with the field trial study (refer to the 860.1380 der for MRID 48728704). Residues of dicamba, DCGA, and DCSA were stable during freezer storage for up to 277 days (9 months) in undelinted cotton seed. The stability of 5-OH dicamba was not determined concurrently because it was not found in undelinted cotton seed. These data are acceptable to support the storage conditions and durations of the undelinted cotton seed samples from the submitted field trials. Storage stability data is not required for cotton gin byproduct samples because the samples were analyzed within 30 days of harvest.

Method validation and concurrent method recovery data are presented in Table C.1. The LC/MS/MS method used to analyze cotton samples for residues of dicamba, 5-OH dicamba, DCGA, and DCSA was adequate for data collection based on acceptable method and concurrent method recovery data. Concurrent recoveries were generally within the acceptable range of 70-120% in cotton fortified with dicamba, 5-OH dicamba, DCGA, and DCSA each at 0.02-5.00 ppm in undelinted cotton seed and 0.04-100.0 ppm in gin byproducts. The fortification levels were adequate to bracket residues found in treated samples. Apparent residues of dicamba, 5-OH dicamba, DCGA, DSGA were below the LOQ (<0.02 ppm or <0.04 ppm) in/on all samples of untreated undelinted cotton seed and cotton gin by-products. Concurrent recoveries were corrected for apparent for residues in controls; residues in treated samples were not corrected for residues in controls.

Residue data from the cotton field trials are reported in Table C.3. A summary of residue data is presented in Table C.4.

Following three broadcast applications of the 4 lb ae/gal SL formulation of dicamba (formulated as the diglycolamine salt), which were applied at pre-emergence, the 6-leaf stage, and first white flower + 15 days for a total rate of 2.0 lb ae/A (2.2 kg ae/ha) (**TRT 2**), combined residues (and per trial averages) of dicamba, 5-OH dicamba, DCGA, and DCSA (expressed in parent equivalents) were <0.08-<0.39 (<0.08-<0.39) ppm in/on undelinted cotton seed collected at PHIs of 49-105 days and <0.67-<2.34 (<0.73-<2.33) ppm in/on gin byproducts collected at PHIs of 82-84 days.

Following three broadcast applications of the 4 lb ae/gal SL formulation of dicamba (formulated as the diglycolamine salt), which were applied at pre-emergence, the first open boll stage, and 7 days prior to harvest for a total rate of 2.0 lb ae/A (2.2 kg ae/ha) (**TRT 3**), combined residues (and per trial averages) of dicamba, 5-OH dicamba, DCGA, and DCSA (expressed in parent equivalents) were <0.12-<2.24 (<0.13-<1.66) ppm in/on undelinted cotton seed collected at a 6-8-day PHI.

Following four foliar broadcast applications of the 4 lb ae/gal SL formulation of dicamba (formulated as the diglycolamine salt), which were applied at the 6-leaf stage, the first white flower + 15 days, the first open boll, and 7 days prior to harvest for a total rate of 2.0-2.1 lb ae/A (2.2-2.4 kg ae/ha) (**TRT 4**), combined residues (and per trial averages) of dicamba, 5-OH dicamba, DCGA, and DCSA (expressed in parent equivalents) were <0.15-<2.02 (<0.21-<1.86) ppm in/on undelinted cotton seed collected at a 6-8-day PHI and <5.50-34.6 (<5.51-<33.7) ppm in/on gin byproducts collected at a 6-7-day PHI. Corresponding combined residues (and per trial averages) in side-by-side trials treated with the 5 lb ae/gal SL formulation of dicamba formulated as the monoethanolamine salt (**TRT 5**) were <0.24-<1.00 (<0.29-<0.82) ppm in/on undelinted cotton seed. Average combined residues in the diglycolamine salt formulation trials were slightly higher (approximately 1.5x) than the corresponding residues in the monoethanolamine salt formulation trials.

Treatment plots 3 and 4 showed the highest average combined residues (0.76 ppm), followed closely by treatment plot 5 (average of 0.52 ppm). Treatment plot 2 had the lowest average combined residues (average of 0.12 ppm). Lower residues from treatment plot 2 is consistent with the earlier application timings at these plots.

For the TRT 2 residue decline samples, average residues of dicamba, 5-OH dicamba, DCGA, and DCSA in/on undelinted cotton seed were at or <LOQ in both studies; therefore, residue decline could not be assessed. For the TRT 4 residue decline samples, average residues of dicamba decreased in/on undelinted cotton seed with increasing PHI. Average residues of DCGA and DCSA increased with increasing PHI in the SC1 trial, and remained relatively constant in the TX1 trial. Residues of 5-OH dicamba were <LOQ in both studies; therefore, residue decline could not be assessed.

TABLE C.1. Summary of Method and Concurrent Recoveries of Dicamba and its Metabolites from Cotton

Matrix	Analyte	Spike Level (ppm)	Sample Size (n)	Recoveries (%)[1]	Mean ± Std. Dev. (%)[2]
Method Validation Recoveries					
Undelinted Cotton seed	Dicamba	0.005	5	187, 73, 172, 105, 102	128 ± 49
		0.010	5	129, 149, 93, 78, 125	115 ± 29
		0.020	5	119, 100, 123, 101, 124	113 ± 12
		0.200	5	94, 96, 95, 97, 100	96.1 ± 2.2
		10.0	5	68, 91, 91, 100, 98	89.6 ± 13

TABLE C.1. Summary of Method and Concurrent Recoveries of Dicamba and its Metabolites from Cotton

Matrix	Analyte	Spike Level (ppm)	Sample Size (n)	Recoveries (%)[1]	Mean ± Std. Dev. (%)[2]
Undelinted Cotton seed (continued)	5-OH Dicamba	0.005	5	68, 56, 101, 84, 105	82.9 ± 21
		0.010	5	106, 97, 76, 110, 82	94.1 ± 15
		0.020	5	96, 98, 115, 117, 114	108 ± 10
		0.200	5	98, 104, 105, 104, 101	102 ± 3.0
		10.0	5	69, 90, 79, 93, 104	87.1 ± 13
	DCGA	0.005	5	140, 115, 88, 98, 129	114 ± 22
		0.010	5	90, 108, 96, 79, 104	95.6 ± 11
		0.020	5	81, 98, 93, 77, 86	86.9 ± 8.5
		0.200	5	90, 87, 88, 84, 96	88.9 ± 4.3
		10.0	5	73, 96, 89, 94, 90	88.6 ± 8.9
	DCSA	0.005	5	88, 95, 90, 134, 98	101 ± 19
		0.010	5	70, 84, 102, 96, 98	89.8 ± 13
		0.020	5	100, 82, 97, 97, 95	94.0 ± 6.9
		0.200	5	95, 100, 105, 96, 101	98.9 ± 4.0
		10.0	5	81, 92, 95, 93, 97	91.6 ± 6.4
Gin Byproducts	Dicamba	0.040	5	100, 93, 88, 122, 119	104 ± 15
		0.400	5	97, 99, 101, 98, 104	100 ± 2.7
		10.0	5	88, 91, 82, 85, 88	86.7 ± 3.1
	5-OH Dicamba	0.040	5	124, 106, 98, 97, 108	106 ± 11
		0.400	5	111, 113, 106, 116, 109	111 ± 3.6
		10.0	5	113, 99, 99, 101, 85	99.4 ± 10
	DCGA	0.040	5	67, 88, 88, 90, 70	80.4 ± 11
		0.400	5	99, 96, 99, 95, 97	97.0 ± 2.0
		10.0	5	101, 94, 105, 99, 104	101 ± 4.4
	DCSA	0.040	5	79, 87, 76, 75, 81	79.5 ± 4.9
		0.400	5	91, 84, 93, 87, 91	89.0 ± 3.7
		10.0	5	110, 101, 92, 109, 99	102 ± 7.6
Concurrent Recoveries					
Undelinted Cotton seed	Dicamba	0.02	19	103, 97.7, 93.5, 104, 104, 98.0, 91.6, 75.5, 96.5, 77.5, 116, 109, 110, 99.9, 99.7, 103, 108, 119, 110	101 ± 11
		0.04	13	105, 106, 84.3, 105, 101, 80.8, 97.6, 119, 114, 110, 100, 108, 109	103 ± 11
		0.20	20	94.5, 105, 97.3, 103, 99.9, 107, 103, 98.5, 102, 95.0, 98.0, 108, 93.9, 97.8, 99.5, 105, 96.5, 101, 105, 94.5	100 ± 4.3
		5.00	1	108	108
	5-OH Dicamba	0.02	13	94.9, 100, 91.0, 79.5, 95.0, 110, 69.5, 68.5, 82.0, 83.0, 88.0, 92.0, 94.0	88.2 ± 12
		0.04	13	93.5, 94.3, 76.5, 86.3, 125, 121, 80.0, 90.3, 105, 110, 109, 107, 92.8	99.3 ± 15
		0.20	15	93.2, 97.5, 97.0, 110, 93.9, 83.7, 115, 86.0, 104, 97.0, 93.5, 94.5, 77.0, 87.5, 108	95.8 ± 10

TABLE C.1. Summary of Method and Concurrent Recoveries of Dicamba and its Metabolites from Cotton

Matrix	Analyte	Spike Level (ppm)	Sample Size (n)	Recoveries (%)[1]	Mean ± Std. Dev. (%)[2]
Undelinted Cotton seed (continued)	DCGA	0.02	16	83.5, 71.4, 93.0, 86.0, 105, 83.5, 107, 101, 114, 120, 116, 87.5, 79.5, 95.5, 93.0, 66.9	93.8 ± 16
		0.04	12	74.0, 83.9, 90.5, 81.3, 77.5, 101, 88.0, 88.8, 93.0, 79.8, 80.5, 80.3	84.8 ± 7.6
		0.20	15	88.0, 92.2, 94.5, 105, 82.5, 86.0, 98.1, 88.0, 90.5, 87.5, 92.5, 77.1, 96.0, 81.5, 100	90.6 ± 7.5
		5.00	1	106	106
	DCSA	0.02	18	105, 101, 100, 94.0, 122, 101, 86.0, 101, 91.0, 98.5, 89.5, 98.0, 103, 106, 101, 92.3, 87.0, 118	99.6 ± 9.4
		0.04	13	84.0, 114, 89.3, 96.3, 108, 95.0, 93.8, 103, 106, 102, 103, 108, 106	101 ± 8.5
		0.20	17	90.5, 100, 95.0, 107, 102, 97.0, 103, 99.5, 103, 101, 92.3, 100, 105, 97.2, 98.2, 101, 103	99.7 ± 4.3
		5.00	1	99.8	99.8
Gin Byproducts	Dicamba	0.04	4	120, 90.5, 88.5, 94.0	98.2 ± 15
		0.40	1	97.2	97.2
		5.00	3	108, 102, 98.6	103 ± 4.8
		100.00	3	96.7, 95.9, 104	98.9 ± 4.5
	5-OH Dicamba	0.04	4	89.7, 96.7, 107, 98.9	98.0 ± 6.9
		0.40	1	103	103
		5.00	3	102, 93.3, 87.3	94.1 ± 7.3
		100.00	3	111, 94.5, 99.2	102 ± 8.5
	DCGA	0.04	4	88.5, 70.0, 93.8, 108	90.1 ± 16
		0.40	1	98.0	98.0
		5.00	3	110, 98.4, 105	105 ± 5.7
		100.00	3	118, 104, 107	110 ± 7.4
	DCSA	0.04	4	88.0, 101, 103, 98.0	97.4 ± 6.6
		0.40	1	91.0	91.0
		5.00	3	125, 114, 123	121 ± 5.9
		100.00	3	99.2, 101, 106	102 ± 3.5

[1] The concurrent recoveries were corrected for apparent residues in the unfortified control samples.
[2] Standard deviation is not applicable for sample sizes n < 3 samples.

TABLE C.2. Summary of Storage Conditions.

Matrix	Analyte	Storage Temperature	Actual Storage Duration[1]	Interval of Demonstrated Storage Stability[2]
Undelinted Cotton seed	Dicamba	Below freezing at field sites -20 °C at analytical lab	13-146 days (0.4-4.8 months)	Residues of dicamba, DCGA, and DCSA are stable during freezer storage for up to 277 days (9 months) in undelinted cotton seed. The stability of 5-OH dicamba was not determined concurrently because it was not detected in undelinted cotton seed.
	5-OH Dicamba		13-122 days (0.4-4.0 months)	
	DCGA		13-285 days (0.4-9.4 months)	
	DCSA		13-121 days (0.4-4.0 months)	
Gin Byproducts	Dicamba	Below freezing at field sites -20 °C at analytical lab	17-27 days (0.6-0.9 months)	Not provided. Storage stability data is not required as the samples were analyzed within 30 days of harvest.
	5-OH Dicamba			
	DCGA			
	DCSA			

[1] Interval from harvest to analysis; all samples were analyzed within 0 to 2 days of extraction.
[2] Refer to the 860.1380 der for MRID 48728704.

TABLE C.3. Residue Data for Dicamba and its Metabolites from Cotton Field Trials

Trial ID (City, County, State; Year)	Zone	Com-modity[1]	Total Rate lb ae/A (kg ae/ha)	TRT[2]	PHI (days)[3]	Residues (ppm) [Average][4, 5]				
						Dicamba	5-OH Dicamba	DCGA	DCSA	Combined Residues
Proctor, Crittenden, AR; 2010 (AR1)	4	Undelinted Cotton seed	2.0 (2.2)	2	70	(0.01), ND [<0.02]	ND, ND [<0.02]	0.03, 0.03 [0.03]	0.05, 0.04 [0.05]	<0.12, <0.11 [<0.12]
				3	7	1.20[6], 0.59[6] [0.89]	ND, ND [<0.02]	0.04, 0.03 [0.03]	0.10, 0.05 [0.08]	<1.37, <0.69 [<1.03]
				4	7	0.33[6], 0.58[6] [0.46]	ND, ND [<0.02]	0.04, 0.05 [0.04]	0.05, 0.07 [0.06]	<0.45, <0.72 [<0.58]
				5	7	0.72, 0.51 [0.62]	ND, ND [<0.02]	0.08, 0.04 [0.06]	0.17, 0.06 [0.12]	<1.00, <0.64 [<0.82]
Porterville, Tulare, CA; 2010 (CA1)	10	Undelinted Cotton seed	2.0 (2.2)	2	81	ND, ND [<0.02]	ND, ND [<0.02]	(0.01), (0.01) [<0.02]	(0.01), (0.01) [<0.02]	<0.08, <0.08 [<0.08]
				3	8	0.18, 0.19 [0.18]	ND, ND [<0.02]	ND, ND [<0.02]	(0.01), 0.02 [<0.02]	<0.24, <0.25 [<0.25]
				4	8	0.47, 0.39 [0.43]	ND, ND [<0.02]	0.04, (0.018) [<0.03]	0.09, 0.04 [0.06]	<0.63, <0.47 [<0.55]
				5	8	0.23, 0.17 [0.20]	ND, ND [<0.02]	0.02, 0.02 [0.02]	0.06, 0.02 [0.04]	<0.34, <0.24 [<0.29]

TABLE C.3. Residue Data for Dicamba and its Metabolites from Cotton Field Trials

Trial ID (City, County, State; Year)	Zone	Com-modity[1]	Total Rate lb ae/A (kg ae/ha)	TRT[2]	PHI (days)[3]	Residues (ppm) [Average][4, 5]				
						Dicamba	5-OH Dicamba	DCGA	DCSA	Combined Residues
Visalia, Tulare, CA; 2010 (CA2)	10	Undelinted Cotton seed	2.0 (2.2)	2	103	ND, ND [<0.02]	ND, ND [<0.02]	(0.01), (0.01) [<0.02]	(0.01), (0.01) [<0.02]	<0.08, <0.08 [<0.08]
				3	8	0.66, 0.64 [0.65]	ND, ND [<0.02]	ND, (0.01) [<0.02]	0.02, 0.05 [0.04]	<0.72, <0.73 [<0.73]
				4	8	1.05, 0.82 [0.94]	ND, ND [<0.02]	0.03, 0.03 [0.03]	0.14, 0.16 [0.15]	<1.25, <1.03 [<1.14]
Chula, Tift, GA; 2010 (GA1)	2	Undelinted Cotton seed	2.0 (2.2)	2	84	ND, ND [<0.02]	ND, ND [<0.02]	(0.01), (0.01) [<0.02]	(0.01), (0.01) [<0.02]	<0.08, <0.08 [<0.08]
				3	7	0.76[6], 1.30[6] [0.90]	ND, ND [<0.02]	(0.01), ND [<0.02]	(0.016), (0.016) [<0.02]	<0.82, <1.36 [<1.09]
				4	7	0.80, 0.71 [0.76]	ND, ND [<0.02]	0.03, 0.02 [0.03]	0.03, 0.02 [0.02]	<0.88, <0.78 [<0.83]
				5	7	0.68, 0.50 [0.59]	ND, ND [<0.02]	0.02, (0.017) [<0.02]	(0.016), (0.016) [<0.02]	<0.74, <0.56 [<0.65]
Cheneyville, Rapides, LA; 2010 (LA1)	4	Undelinted Cotton seed	2.0 (2.2)	2	73	ND, ND [<0.02]	ND, ND [<0.02]	(0.01), (0.01) [<0.02]	(0.01), (0.01) [<0.02]	<0.08, <0.08 [<0.08]
				3	7	0.33, 0.45 [0.39]	ND, ND [<0.02]	0.02, (0.019) [<0.02]	(0.01), (0.01) [<0.02]	<0.39, <0.51 [<0.45]
				4	7	0.82, 0.65 [0.74]	ND, ND [<0.02]	0.03, 0.03 [0.03]	0.03, 0.06 [0.04]	<0.90, <0.76 [<0.83]
Fisk, Butler, MO; 2010 (MO1)	4	Undelinted Cotton seed	2.0 (2.2)	2	79	ND, ND [<0.02]	ND, ND [<0.02]	(0.015), (0.015) [<0.02]	0.02, (0.017) [<0.02]	<0.08, <0.08 [<0.08]
				3	7	0.97, 1.00 [0.98]	ND, ND [<0.02]	(0.019), (0.01) [<0.02]	(0.01), 0.06 [<0.03]	<1.03, <1.10 [<1.07]
				4	7	0.56[6], 1.17[6] [0.87]	ND, ND [<0.02]	0.02, 0.02 [0.02]	0.02, 0.03 [0.03]	<0.62, <1.25 [<0.94]

TABLE C.3. Residue Data for Dicamba and its Metabolites from Cotton Field Trials

Trial ID (City, County, State; Year)	Zone	Com-modity[1]	Total Rate lb ae/A (kg ae/ha)	TRT[2]	PHI (days)[3]	Residues (ppm) [Average][4, 5]				
						Dicamba	5-OH Dicamba	DCGA	DCSA	Combined Residues
Hinton, Caddo, OK; 2010 (OK1)	8	Undelinted Cotton seed	2.0 (2.2)	2	68	ND, ND [<0.02]	ND, ND [<0.02]	0.03, 0.03 [0.03]	0.03, 0.03 [0.03]	<0.10, <0.10 [<0.10]
				3	8	0.33, 0.23 [0.28]	ND, ND [<0.02]	0.05, 0.03 [0.04]	0.08, 0.03 [0.06]	<0.49, <0.31 [<0.40]
				4	8	0.35[6], 0.18[6] [0.27]	ND, ND [<0.02]	0.09, 0.05 [0.07]	0.11, 0.07 [0.09]	<0.57, <0.33 [<0.45]
				5	8	0.23, 0.23 [0.23]	ND, ND [<0.02]	0.03, 0.04 [0.04]	0.02, 0.07 [0.04]	<0.30, <0.37 [<0.33]
Dill City, Washita, OK; 2010 (OK2)	8	Undelinted Cotton seed	2.0 (2.2)	2	82	ND, ND [<0.02]	ND, ND [<0.02]	0.03, 0.02 [0.03]	0.03, (0.01) [<0.02]	<0.10, <0.08 [<0.09]
				3	7	0.07, 0.06 [0.06]	ND, ND [<0.02]	ND, ND [<0.02]	(0.01), (0.01) [<0.02]	<0.13, <0.12 [<0.13]
				4	7	0.12, 0.16 [0.14]	ND, ND [<0.02]	0.02, 0.03 [0.03]	0.03, 0.03 [0.03]	<0.19, <0.24 [<0.22]
		Gin byproducts		2	82	ND, ND [<0.04]	(0.01), (0.01) [<0.04]	0.17, 0.23 [0.20]	0.39, 0.46 [0.43]	<0.67, <0.80 [<0.73]
				4	7	3.17, 3.09 [3.13]	(0.01), (0.01) [<0.04]	0.49, 0.40 [0.45]	1.70, 1.85 [1.78]	<5.52, <5.50 [<5.51]
Elko, Barnwell, SC; 2010 (SC1)	2	Undelinted Cotton seed	2.0 (2.2)	2	99	ND, ND [<0.02]	ND, ND [<0.02]	(0.01), (0.01) [<0.02]	(0.01), (0.01) [<0.02]	<0.08, <0.08 [<0.08]
					105	ND, ND [<0.02]	ND, ND [<0.02]	(0.01), (0.01) [<0.02]	(0.01), (0.01) [<0.02]	<0.08, <0.08 [<0.08]
					112	ND, ND [<0.02]	ND, ND [<0.02]	(0.01), (0.01) [<0.02]	(0.01), (0.01) [<0.02]	<0.08, <0.08 [<0.08]
					119	ND, ND [<0.02]	ND, ND [<0.02]	(0.01), (0.01) [<0.02]	(0.01), (0.01) [<0.02]	<0.08, <0.08 [<0.08]
				3	7	0.18, 0.06 [0.12]	ND, ND [<0.02]	ND, 0.02 [<0.02]	(0.01), 0.02 [<0.02]	<0.24, <0.12 [<0.18]
				4	1	0.21, 0.31 [0.26]	ND, ND [<0.02]	0.02, 0.03 [0.03]	0.02, 0.03 [0.03]	<0.28, <0.39 [<0.34]
					7	0.14, 0.09 [0.12]	ND, ND [<0.02]	0.06, (0.018) [<0.04]	0.05, 0.02 [0.04]	<0.27, <0.15 [<0.21]

TABLE C.3. Residue Data for Dicamba and its Metabolites from Cotton Field Trials

Trial ID (City, County, State; Year)	Zone	Com-modity[1]	Total Rate lb ae/A (kg ae/ha)	TRT[2]	PHI (days)[3]	Residues (ppm) [Average][4, 5]				
						Dicamba	5-OH Dicamba	DCGA	DCSA	Combined Residues
					14	0.09, 0.07 [0.08]	ND, ND [<0.02]	0.10[7], 0.05[7] [0.05]	0.06, 0.03 [0.05]	<0.28, <0.17 [<0.22]
					21	0.04, 0.08 [0.06]	ND, ND [<0.02]	0.11[7], 0.04[7] [0.06]	0.09, 0.03 [0.06]	<0.26, <0.17 [<0.22]
Raymondville, Willacy, TX; 2010 (TX1)	6	Undelinted Cotton seed	2.0 (2.2)	2	43	ND, ND [<0.02]	ND, ND [<0.02]	0.02, (0.019) [<0.02]	0.03, (0.01) [<0.02]	<0.09, <0.08 [<0.09]
					49	ND, ND [<0.02]	ND, ND [<0.02]	(0.01), 0.02 [<0.02]	0.02, (0.01) [<0.02]	<0.08, <0.08 [<0.08]
					56	ND, ND [<0.02]	ND, ND [<0.02]	(0.01), (0.019) [<0.02]	(0.01), 0.03 [<0.02]	<0.08, <0.09 [<0.08]
					63	ND, ND [<0.02]	ND, ND [<0.02]	(0.018), (0.01) [<0.02]	(0.016), (0.01) [<0.02]	<0.08, <0.08 [<0.08]
				3	7	0.58[6], 0.19[6] [0.38]	ND, ND [<0.02]	ND, ND [<0.02]	0.07, (0.01) [<0.04]	<0.70, <0.25 [<0.48]
	6	Undelinted Cotton seed	2.1 (2.4)	4	1	0.28, 0.23 [0.25]	ND, ND [<0.02]	0.03, 0.03 [0.03]	0.03, 0.03 [0.03]	<0.36, <0.31 [<0.33]
					7	0.19, 0.17 [0.18]	ND, ND [<0.02]	0.03, 0.02 [0.03]	0.09, 0.04 [0.06]	<0.34, <0.25 [<0.29]
					14	0.07, 0.04 [0.05]	ND, ND [<0.02]	0.03, 0.02 [0.03]	0.04, 0.03 [0.04]	<0.17, <0.11 [<0.14]
					21	0.03, 0.04 [0.03]	ND, ND [<0.02]	0.03, 0.03 [0.03]	0.03, 0.03 [0.03]	<0.12, <0.13 [<0.12]
Levelland, Hockley, TX; 2010 (TX2)	8	Undelinted Cotton seed	2.0 (2.2)	2	84	ND, ND [<0.02]	ND, ND [<0.02]	0.11, 0.11 [0.11]	0.23, 0.23 [0.23]	<0.39, <0.39 [<0.39]
				3	6	0.84[6], 1.31[6] [1.07]	(0.01), ND [<0.02]	0.07, 0.05 [0.06]	0.07[6], 0.25[6] [0.16]	<1.00, <1.64 [<1.32]
				4	6	1.30, 1.54 [1.42]	ND, (0.01) [<0.02]	0.11, 0.17 [0.14]	0.26, 0.27 [0.27]	<1.71, <2.02 [<1.86]
		Gin byproducts		2	84	ND, (0.01) [<0.04]	ND, (0.01) [<0.04]	0.42, 0.71 [0.57]	1.73, 1.43 [1.58]	<2.34, <2.31 [<2.33]
				4	6	16.4, 13.4 [14.9]	0.04, (0.03) [<0.04]	3.25, 1.56 [2.41]	6.02, 2.97 [4.50]	<26.1, <18.2 [<22.1]

TABLE C.3. Residue Data for Dicamba and its Metabolites from Cotton Field Trials

Trial ID (City, County, State; Year)	Zone	Com-modity[1]	Total Rate lb ae/A (kg ae/ha)	TRT[2]	PHI (days)[3]	Residues (ppm) [Average][4, 5]				
						Dicamba	5-OH Dicamba	DCGA	DCSA	Combined Residues
Wolfforth, Lubbock, TX; 2010 (TX3)	8	Undelinted Cotton seed	2.0 (2.2)	2	84	ND, ND [<0.02]	ND, ND [<0.02]	0.07, 0.04 [0.05]	0.10, 0.04 [0.07]	<0.22, <0.12 [<0.17]
				3	6	1.97[6], 0.80[6] [1.38]	(0.01), ND [<0.02]	0.07, 0.10 [0.09]	0.16, 0.16 [0.16]	<2.24, <1.09 [<1.66]
				4	6	1.29, 1.08 [1.19]	ND, ND [<0.02]	0.08, 0.12 [0.10]	0.11, 0.16 [0.14]	<1.51, <1.39 [<1.45]
		Gin byproducts		2	84	ND, ND [<0.04]	(0.01), (0.01) [<0.04]	0.32, 0.31 [0.32]	0.85, 0.49 [0.67]	<1.30, <0.91 [<1.11]
				4	6	23.6, 22.4 [23.0]	(0.03), (0.03) [<0.04]	4.31, 3.97 [4.14]	6.29, 6.05 [6.17]	<34.6, <32.8 [<33.7]
Uvalde, Uvalde, TX; 2010 (TX4)	8	Undelinted Cotton seed	2.0 (2.2)	2	71	ND, ND [<0.02]	ND, ND [<0.02]	(0.01), (0.01) [<0.02]	(0.01), (0.01) [<0.02]	<0.08, <0.08 [<0.08]
				3	6	0.86, 1.22 [1.04]	ND, (0.016) [<0.02]	0.02, 0.03 [0.03]	0.03, 0.04 [0.03]	<0.93, <1.31 [<1.12]
				4	6	0.49, 0.44 [0.47]	ND, ND [<0.02]	0.03, 0.03 [0.03]	0.03, 0.04 [0.04]	<0.58, <0.53 [<0.55]

[1] Each trial site was planted with Event MON 88701 cotton that is tolerant to dicamba herbicide and glufosinate-ammonium herbicide.

[2] TRT 2: Applications with 4 lb ae/gal SL formulation made at preemergence, the 6-leaf stage, and at the first white flower + 15 days stage; TRT 3: Applications with 4 lb ae/gal SL formulation made at preemergence, the first open boll stage, and at 7 days prior to harvest; TRT 4: Applications with 4 lb ae/gal SL formulation made at the 6-leaf stage, the first white flower + 15-days stage, the first open boll stage, and at 7 days prior to harvest; TRT 5: Applications with 5 lb ae/gal SL formulation made at the 6-leaf stage, the first white flower + 15-days stage, the first open boll stage, and at 7 days prior to harvest.

[3] For the residue decline trials, the target PHI is **bolded**.

[4] ND = <LOD. The LODs for the analytes in undelinted cotton seed were 0.0035, 0.0041, 0.0057 and 0.0089 ppm for DCSA, DCGA, 5-hydroxydicamba and dicamba, respectively. The LOQ was 0.02 ppm for each analyte in undelinted cotton seed and 0.04 ppm in cotton gin byproducts. Values between the LOQ and LOD are in parenthesis.

[5] Residues for individual analytes are presented as per se. Combined residues of dicamba, 5-OH dicamba, DCGA, and DCSA are expressed in parent equivalents. Residues of 5-OH dicamba, DCSA, and DCGA were converted to parent equivalents by the study reviewer using molecular weight conversion factors of 0.933, 1.068, and 0.991, respectively. When calculating per trial averages, the LOQ was used for values reported as ND or between the LOD and LOQ. **NOTE TO EPA REVIEWER: DCGA is included in the combined residues as it is a major metabolite of dicamba.**

[6] Average of multiple analyses.

[7] The data in Table 13 (p. 53) does not match the raw data in the Master Summary Table (p. 361). It appears that the DCGA data in Table 13 was copied from the DCSA data. The study reviewer used the raw data for DCGA.

TABLE C.4. Summary of Residue Data from Cotton Crop Field Trials with Dicamba.

Commodity	Analyte	Total App. Rate lb ae/A (kg ae/ha)	PHI (days)	Residue Levels (ppm)[1]							
				n	Sample Min.	Sample Max.	LAFT[2]	HAFT[2]	Median	Mean	Std. Dev.
TRT 2 (Applications at Preemergence, 6-leaf stage, and first white flower + 15 days; EP: Clarity)											
Undelinted Cotton seed	Dicamba	2.0 (2.2)	49-105	13	<0.02	<0.02	<0.02	<0.02	0.02	0.02	N/A
	5-OH Dicamba			13	<0.02	<0.02	<0.02	<0.02	0.02	0.02	N/A
	DCGA			13	<0.02	0.11	<0.02	0.11	0.02	0.03	0.03
	DCSA			13	<0.02	0.23	<0.02	0.23	0.02	0.04	0.06
	Combined Residues			13	<0.08	<0.39	<0.08	<0.39	0.08	0.12	0.09
Gin byproducts	Dicamba	2.0 (2.2)	82-84	3	<0.04	<0.04	<0.04	<0.04	0.04	0.04	N/A
	5-OH Dicamba			3	<0.04	<0.04	<0.04	<0.04	0.04	0.04	N/A
	DCGA			3	0.17	0.71	0.20	0.57	0.32	0.36	0.19
	DCSA			3	0.39	1.73	0.43	1.58	0.67	0.89	0.61
	Combined Residues			3	<0.67	<2.34	<0.73	<2.33	1.11	1.39	0.84
TRT 3 (Applications at Preemergence, first open boll stage, and 7 days prior to harvest; EP: Clarity)											
Undelinted Cotton seed	Dicamba	2.0 (2.2)	6-8	13	0.06	1.97	0.06	1.38	0.65	0.64	0.43
	5-OH Dicamba			13	<0.02	0.02	<0.02	<0.02	0.02	0.02	N/A
	DCGA			13	<0.02	0.10	<0.02	0.09	0.02	0.03	0.02
	DCSA			13	<0.02	0.25	<0.02	0.16	0.03	0.05	0.05
	Combined Residues			13	<0.12	<2.24	<0.13	<1.66	0.73	0.76	0.49
TRT 4 (Applications at 6-leaf, first white flower + 15 days, first open boll, and 7 days prior to harvest; EP: Clarity)											
Undelinted Cotton seed	Dicamba	2.0-2.1 (2.2-2.4)	6-8	13	0.09	1.54	0.12	1.42	0.47	0.61	0.41
	5-OH Dicamba			13	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	N/A
	DCGA			13	0.02	0.17	0.02	0.14	0.03	0.05	0.04
	DCSA			13	0.02	0.27	0.02	0.27	0.06	0.08	0.07
	Combined Residues			13	<0.15	<2.02	<0.21	<1.86	0.58	0.76	0.49
Gin byproducts	Dicamba	2.0 (2.2)	6-7	3	3.09	23.6	3.13	23.0	14.9	13.7	10.0
	5-OH Dicamba			3	<0.04	0.04	<0.04	<0.04	0.04	0.04	N/A
	DCGA			3	0.40	4.31	0.45	4.14	2.41	2.33	1.85
	DCSA			3	1.70	6.29	1.78	6.17	4.50	4.15	2.22
	Combined Residues			3	<5.50	34.6	<5.51	<33.7	22.1	20.4	14.2
TRT 5 (Applications at 6-leaf, first white flower + 15 days, first open boll, and 7 days prior to harvest; EP: MON 11968)											
Undelinted Cotton seed	Dicamba	2.0 (2.2)	7-8	4	0.17	0.72	0.20	0.62	0.41	0.41	0.23
	5-OH Dicamba			4	<0.02	<0.02	<0.02	<0.02	0.02	0.02	N/A
	DCGA			4	0.02	0.08	0.02	0.06	0.03	0.04	0.02
	DCSA			4	0.02	0.17	0.02	0.12	0.04	0.06	0.04
	Combined Residues			4	<0.24	<1.00	<0.29	<0.82	0.49	0.52	0.26

[1] Except for sample min/max, values reflect per trial averages; n = no. of field trials. For calculation of median, mean, and standard deviation, the LOQ (0.02 ppm each analyte in undelinted cotton seed and 0.04 ppm for each analyte in cotton gin

byproducts) was used for any results reported as <LOQ in Table C.3. Combined residues of dicamba, 5-OH dicamba, DCGA, and DCSA are expressed in parent equivalents. Individual analyte results are reported as per se. **NOTE TO EPA REVIEWER: DCGA is included in the combined residues as it is a major metabolite of dicamba.**
N/A = Not applicable.
[2] LAFT = lowest-average-field-trial; HAFT = highest-average-field-trial.

D. CONCLUSION

The submitted cotton field trial data are adequate and reflect the use of three to four broadcast applications of the 4 lb ae/gal SL formulation of dicamba (formulated as the diglycolamine salt), at a total rate of 2.0-2.1 lb ae/A (2.2-2.4 kg ae/ha). Following three applications at pre-emergence, the 6-leaf stage, and first white flower + 15 days **(TRT2)**, combined residues (and per trial averages) of dicamba, 5-OH dicamba, DCGA, and DCSA (expressed in parent equivalents) were <0.08-<0.39 (<0.08-<0.39) ppm in/on undelinted cotton seed collected at PHIs of 49-105 days and <0.67-<2.34 (<0.73-<2.33) ppm in/on gin byproducts collected at PHIs of 82-84 days. Following three applications at pre-emergence, the first open boll stage, and 7 days prior to harvest **(TRT 3)** combined residues (and per trial averages) of dicamba, 5-OH dicamba, DCGA, and DCSA (expressed in parent equivalents) were <0.12-<2.24 (<0.13-<1.66) ppm in/on undelinted cotton seed collected at a 6-8-day PHI. Following four applications at the 6-leaf stage, the first white flower + 15 days, the first open boll, and 7 days prior to harvest **(TRT 4)**, combined residues (and per trial averages) of dicamba, 5-OH dicamba, DCGA, and DCSA (expressed in parent equivalents) were <0.15-<2.02 (<0.21-<1.86) ppm in/on undelinted cotton seed collected at a 6-8-day PHI and <5.50-34.6 (<5.51-<33.7) ppm in/on gin byproducts collected at a 6-7-day PHI. Corresponding combined residues (and per trial averages) in side-by-side trials treated with the 5 lb ae/gal SL formulation of dicamba formulated as the monoethanolamine salt (**TRT 5**) were <0.24-<1.00 (<0.29-<0.82) ppm in/on undelinted cotton seed. Average combined residues in the diglycolamine salt formulation trials were slightly higher (approximately 1.5x) than the corresponding residues in the monoethanolamine salt formulation trials.

Treatment plots 3 and 4 showed the highest average combined residues (0.76 ppm), followed closely by treatment plot 5 (average of 0.52 ppm). Treatment plot 2 had the lowest average combined residues (average of 0.12 ppm). Lower residues from treatment plot 2 is consistent with the earlier application timings at these plots.

For the TRT 2 residue decline samples, average residues of dicamba, 5-OH dicamba, DCGA, and DCSA in/on undelinted cotton seed were at or <LOQ in both studies; therefore, residue decline could not be assessed. For the TRT 4 residue decline samples, average residues of dicamba decreased in/on undelinted cotton seed with increasing PHI. Average residues of DCGA and DCSA increased with increasing PHI in the SC1 trial, and remained relatively constant in the TX1 trial. Residues of 5-OH dicamba were <LOQ in both studies; therefore, residue decline could not be assessed.

An acceptable method was used for residue quantitation, and adequate data were submitted to support sample storage intervals and conditions.

E. REFERENCES

None.

F. DOCUMENT TRACKING

Petition Number(s): 2F8067
DP Barcode(s): 408384
PC Code: 029801, 029802, 029806, 128931, 128944, & 129043

Template Version June 2005.

Primary Evaluator		Date: 11/15/2012
	Versar, Inc.	
Approved by	Peter Savoia	Date: 04/22/2013
	Peter Savoia, Chemist, OCSPP-HED-RAB V/VII	

Note: This DER was originally prepared under contract by Versar, Inc. (6850 Versar Center, Springfield, VA 22151; submitted 11/15/12). The DER has been reviewed by the Health Effects Division (HED) and revised to reflect current Office of Pesticide Programs (OPP) policies.

STUDY REPORTS:

48728703 Maher, D.; Foster, J. (2011) Magnitude of Dicamba Residues in Cotton Raw Agricultural and Processed Commodities following Applications of Dicamba-Based Formulations to MON 88701. Project Number: REG/10/062/OCR, MSL0024072. Unpublished study prepared by Monsanto Company, Carringers, Inc. and GLP Technologies. 603 p.

EXECUTIVE SUMMARY:

Monsanto has submitted a processing study for dicamba on dicamba-tolerant cotton, identified as Event MON 88701 (formerly GH_S26695). Event MON 88701 cotton expresses a modified dicamba mono-oxygenase (DMO) gene derived from *Stenotrophomonas maltophilia* and the bialaphos resistance (BAR) gene isolated from *Streptomyces hygroscopicus*. The expression of DMO confers dicamba tolerance and the expression of BAR confers glufosinate tolerance to Event MON 88701 cotton. Two trials were conducted in MO and TX during the 2010 growing season.

Each trial contained two treated plots that followed one of two treatment types: three broadcast applications applied at pre-emergence, 6-leaf, and 1st white flower + 15 days [TRT 2] or four foliar broadcast applications applied at 6-leaf, 1st white flower + 15 days, first open boll, and 7 days prior to harvest [TRT 4]. Applications were made at the field rate using a 4 lb ae/gal water soluble concentrate (SL) formulation of dicamba diglycolamine salt (Clarity Herbicide; MON 54140). The total application rate for both treatment types was 2.0 lb ae/A (2.2 kg ae/ha). For TRT 2, application 1 was applied at 1.0 lb ae/A (1.1 kg ae/ha) and applications 2 and 3 were applied at 0.50 lb ae/A/application (0.56 kg ae/ha/application). Retreatment intervals (RTIs) were 24-49 days between the first and second applications and 42-47 days between the second and third applications. For TRT 4, all four applications were applied at 0.49-0.51 lb ae/A/application (0.55-0.57 kg ae/ha/application). RTIs were 42-47 days between the first and second applications, 36-42 days between the second and third applications, and 29-30 days between the third and fourth applications.

All applications were made using ground equipment in spray volumes of 19.7-20.5 gal/A (184-192 l/ha) and included a nonionic surfactant (NIS) and ammonium sulfate (AMS) at all trial sites. Samples of undelinted cotton seed were harvested using a mechanical picker at maturity (preharvest interval [PHI] of 71-79 days for TRT 2 and 6-7 days for TRT 4). Samples of cotton

seed were processed into meal, hulls, alkali-refined oil, and RBD oil by GLP Technologies (Navasota, TX) using procedures simulating commercial processing.

Samples were analyzed for residues of dicamba and metabolites 5-hydroxydicamba (5-OH dicamba), 3,6-dichlorosalicylic acid (DCSA), and 3,6-dichlorogentisic acid (DCGA) using a high performance liquid chromatography method with tandem mass spectrometry detection (LC/MS/MS). The limit of quantitation (LOQ, equivalent to the lowest level of method validation; LLMV) was 0.02 ppm for all analytes in undelinted cotton seed (RAC) and the processed commodities. The method was adequate for data validation based on acceptable method and concurrent recovery data. The fortification levels used in concurrent method recovery were adequate to bracket expected residue levels. Concurrent recoveries were corrected for apparent residues in controls; residues in treated samples were not corrected for residues in controls. We note that the analytical method does not specify conversion of metabolite residues to parent equivalents; therefore, quantifiable residues of 5-OH dicamba, DCSA, and DCGA were converted to parent equivalents by the study reviewer using a molecular weight conversion factor of 0.933, 1.068, and 0.991, respectively.

Samples were stored frozen (~-20 °C) from harvest/processing to analysis for 152-196 days (5.0-6.4 months) for cotton (RAC) and 19-40 days (0.6-1.3 months) for the processed commodities. All samples were analyzed on the day of extraction. Processing took place within 118-169 days of harvest. A storage stability study for dicamba, DCGA, and DCSA on undelinted cotton seed was submitted in conjunction with the field trial study (refer to the 860.1380 der for MRID 48728704). The stability of 5-OH dicamba was not determined concurrently because it was not found in undelinted cotton seed. These data are acceptable to support the storage conditions and durations of the undelinted cotton seed samples from the submitted field trials. Storage stability data for the processed commodities was not provided; however, storage stability data is not required as the processed commodities were generally analyzed within 30 days of processing.

Following three or four applications of the 4 lb ae/gal SC formulation at a total rate of 2 lb ae/A (2.2 kg ae/ha), using PHIs of 71-79 days and 6-7 days, respectively, the combined residues of dicamba, 5-OH dicamba, DCGA, and DCSA (in parent equivalents) were below the LOQ (<0.08)-<0.78 ppm in/on undelinted cotton seed (RAC), <0.081-<0.79 ppm in hulls, below the LOQ (<0.08)-<0.28 ppm in meal, and below the LOQ (<0.08 ppm) in alkali refined oil and RBD oil.

The processing data indicate that the combined residues do not concentrate in hulls, meal, alkali refined oil, or RBD oil (average processing factors of 0.86x, 0.49, 0.11x, and 0.11x, respectively).
The processing factors calculated in this study for combined residues of dicamba, 5-OH dicamba, DCGA, and DCSA in cotton were less than the theoretical concentration factor of 3.8x for hulls, 2.2x for meal, and 6.3x for oil (based separation of components; OPPTS 860.1520, Table 3).

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the field trial residue data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document D408384.

COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance, and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Dicamba (3,6-dichloro-2-methoxybenzoic acid is a selective benzoic acid herbicide registered for the control of weeds prior to their emergence. The Dicamba Reregistration Eligibility Decision (RED) was issued December 2005. The chemical structure and nomenclature of dicamba and its metabolites 5-OH dicamba, DSCA, and DCGA are presented in Table A.1. The physicochemical properties of the technical grade of dicamba acid are presented in Table A.2.

TABLE A.1. Test Compound Nomenclature.

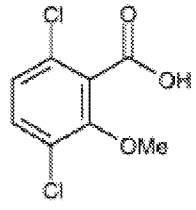
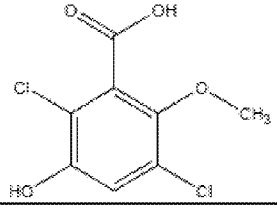
Compound	
Common name	Dicamba
Company experimental name	MON 11900
IUPAC name	3,6-dichloro-o-anisic acid or 3,6-dichloro-2-methoxybenzoic acid
CAS name	3,6-dichloro-2-methoxybenzoic acid
CAS registry number	1918-00-9 (dicamba acid), 104040-79-1 (diglycolamine salt), or 53404-28-7 (monoethanolamine salt)
End-use product	Clarity® Herbicide: SL formulation containing 4 lb ae/gal
Compound	
Common name	5-Hydroxy-dicamba
Company experimental name	5-OH dicamba
IUPAC/CAS name	2,5-dichloro-3-hydroxy-6-methoxybenzoic acid
CAS registry number	7600-50-2

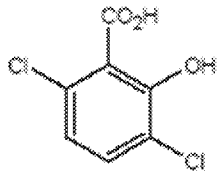
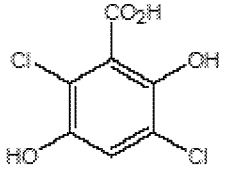
TABLE A.1. Test Compound Nomenclature.	
Compound	
Common name	DCSA; 3,6-dichlorosalicylic acid
Company experimental name	MON 52708
IUPAC/CAS name	3,6-dichloro-2-hydroxybenzoic acid
CAS registry number	3401-80-7
Compound	
Common name	DCGA; 3,6-dichlorogentistic acid
Company experimental name	MON 52724
IUPAC/CAS name	2,5-dichloro-3,6-dihydroxybenzoic acid
CAS registry number	18688-01-2

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound: Dicamba.		
Parameter	Value	Reference
Melting point	114-116 EC (PAI) 90-100 EC (87% TGAI)	Residue Chemistry Chapter of the Dicamba RED, DP# 317699, 12/20/05, C. Olinger
pH	2.5-3.0 (87% TGAI)	
Density	1.57 g/mL at 25 EC (87% TGAI)	
Water solubility	0.5 g/100 mL at 25 EC (PAI)	
Solvent solubility	g/100 mL at 25 EC (PAI) dioxane 118.0 ethanol 92.2 isopropyl alcohol 76.0 methylene chloride 26.0 acetone 17.0 toluene 13.0 xylene 7.8 heavy aromatic naphthalene 5.2	
Vapor pressure	3.4 x 10^{-5} mm Hg at 25 EC (PAI)	
Dissociation constant, pK_a	1.97 (PAI)	
Octanol/water partition coefficient, Log(K_{OW})	0.1 (PAI)	
UV/visible absorption spectrum	neutral: 511 (275 nm) acidic (pH 0-1): 1053 (281 nm) basic (pH 13-14): 469 (274 nm)	

B. EXPERIMENTAL DESIGN

B.1. Application and Crop Information

In two crop field trials conducted in MO and TX, one untreated and two treated plots were planted with dicamba-tolerant cotton (Event MON 88701) containing a dicamba mono-oxygenase (DMO) gene to confer tolerance to dicamba and a bialophos resistance (BAR) gene to confer tolerance to the glufosinate. Each treated plot followed one of two treatment types: three broadcast applications applied at pre-emergence, 6-leaf, and 1st white flower + 15 days [TRT 2] or four foliar broadcast applications applied at 6-leaf, 1st white flower + 15 days, first open boll, and 7 days prior to harvest [TRT 4]. Applications were made at the field rate using a 4 lb ae/gal SL formulation of dicamba diglycolamine salt (Clarity Herbicide; MON 54140). The total application rate for both treatment types was 2.0 lb ae/A (2.2 kg ae/ha). For TRT 2, application 1 was applied at 1.0 lb ae/A (1.1 kg ae/ha) and applications 2 and 3 were applied at 0.50 lb ae/A/application (0.56 kg ae/ha/application). RTIs were 24-49 days between the first and second applications and 42-47 days between the second and third applications. For TRT 4, all four applications were applied at 0.49-0.51 lb ae/A/application (0.55-0.57 kg ae/ha/application). RTIs were 42-47 days between the first and second applications, 36-42 days between the second and third applications, and 29-30 days between the third and fourth applications.

All applications were made using ground equipment in spray volumes of 19.7-20.5 gal/A (184-192 l/ha) and included an NIS and an AMS at all trial sites. Samples of undelinted cotton seed were harvested using a mechanical picker at maturity (PHI of 71-79 days for TRT2 and 6-7 days for TRT4). Study use pattern data are reported in Table B.1.

TABLE B.1. Study Use Pattern.

Location City, County, State;Year (Trial ID)	EP[1]	TRT	Application					Tank Mix/ Adjuvants[3]	Harvest Procedures
			Method; Timing	Volume gal/A (l/ha)	Rate lb ae/A (kg ae/A)	RTI[2] (days)	Total Rate lb ae/A (kg ae/A)		
Fisk, Butler, MO; 2010 (MO1)	4 lb ae/gal SL	2	1. Broadcast; Preemergence	20.0 (187)	1.0 (1.1)	--	2.0 (2.2)	NIS + AMS	Mechanical picker
			2. Foliar broadcast; 6-leaf	20.0 (187)	0.50 (0.56)	29			
			3. Foliar broadcast; 1st WF + 15 days	20.0 (187)	0.50 (0.56)	42			
	4 lb ae/gal SL	4	1. Foliar broadcast; 6-leaf	20.3 (190)	0.51 (0.57)	--	2.0 (2.2)	NIS + AMS	Mechanical picker
			2. Foliar broadcast; 1st WF + 15 days	20.1 (188)	0.50 (0.56)	42			
			3. Foliar broadcast; 1st open boll	20.0 (187)	0.50 (0.56)	42			
			4. Foliar broadcast; 7 days prior to harvest	20.0 (187)	0.50 (0.56)	30			

TABLE B.1. Study Use Pattern.

Location City, County, State;Year (Trial ID)	EP[1]	TRT	Application					Tank Mix/ Adjuvants[3]	Harvest Procedures
			Method; Timing	Volume gal/A (l/ha)	Rate lb ae/A (kg ae/A)	RTI[2] (days)	Total Rate lb ae/A (kg ae/A)		
Uvalde, Uvalde, TX; 2010 (TX4)	4 lb ae/gal SL	2	1. Broadcast; Preemergence	20.2 (189)	1.0 (1.1)	--	2.0 (2.2)	NIS + AMS	Mechanical picker
			2. Foliar broadcast; 6-leaf	20.1 (188)	0.50 (0.56)	49			
			3. Foliar broadcast; 1st WF + 15 days	19.9 (186)	0.50 (0.56)	47			
	4 lb ae/gal SL	4	1. Foliar broadcast; 6-leaf	20.0 (187)	0.50 (0.56)	--	2.0 (2.2)	NIS + AMS	Mechanical picker
			2. Foliar broadcast; 1st WF + 15 days	19.7 (184)	0.49 (0.55)	47			
			3. Foliar broadcast; 1st open boll	20.5 (192)	0.49 (0.55)	36			
			4. Foliar broadcast; 7 days prior to harvest	20.2 (189)	0.51 (0.57)	29			

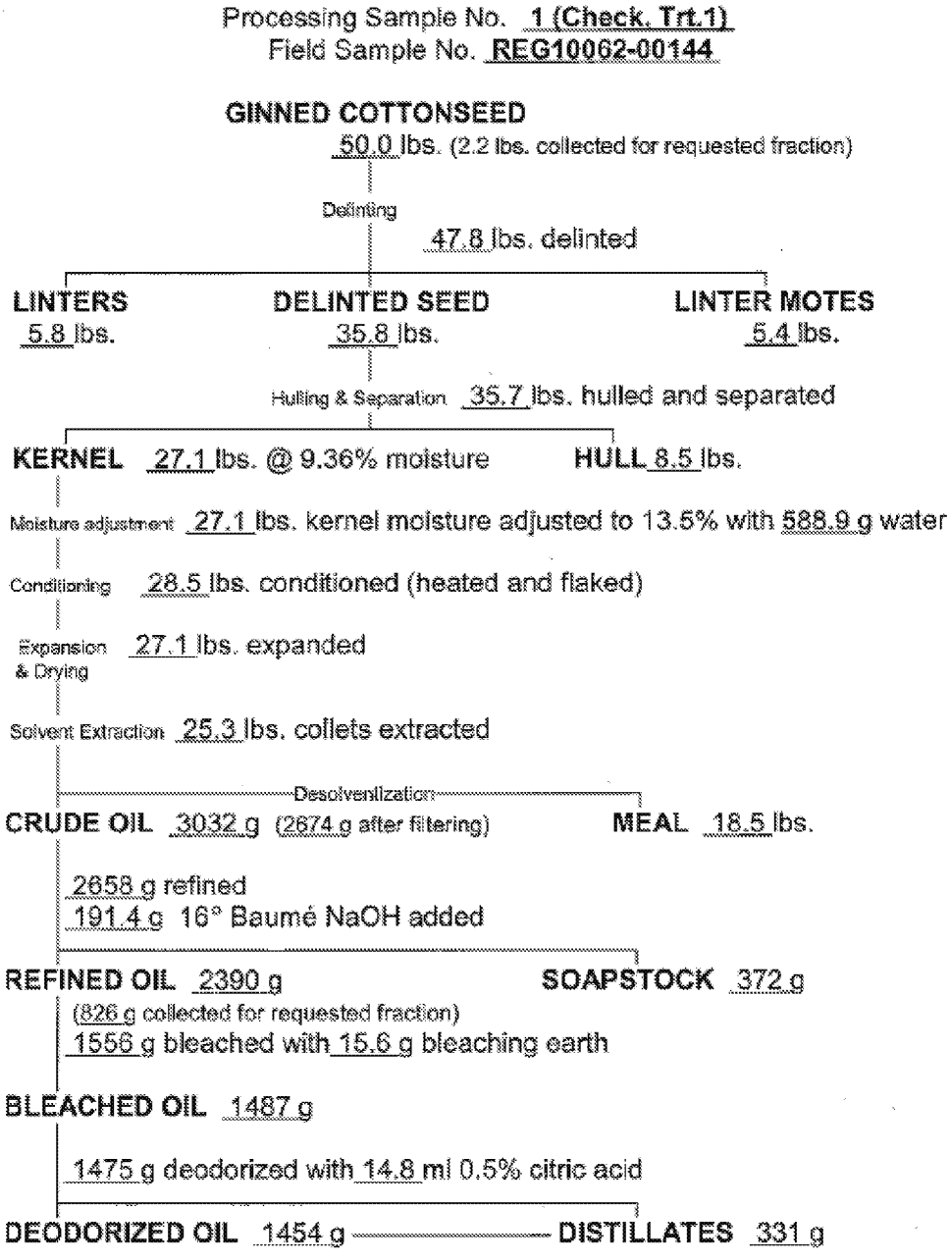
[1] EP = End-use Product; Clarity [water soluble concentrate (SL) formulation containing 480 g/L dicamba formulated as the diglycolamine salt (4 lb ae/gal)].
[2] Retreatment Interval.
[3] NIS = nonionic surfactant. AMS = ammonium sulfate.

B.2. Sample Handling and Processing Procedures

Single bulk samples of undelinted cotton seed (~19-32 kg) from the control and treated plots were collected and ginned at the field sites within 3 hours of harvest. Samples were placed in frozen storage within 1.0 hour of collection. The samples were stored frozen for 5-25 days after collection then shipped frozen via freezer truck to the processing facility, GLP Technologies (Navasota, TX). Cotton seed samples were stored frozen (~<-12 °C) at GLP Technologies until processing and were processed within 118-169 days of harvest. Samples from both trials were processed into meal, hulls, alkali-refined oil, and RBD oil using procedures simulating commercial processing. Samples were stored frozen after processing and were shipped frozen within 6-20 days after processing via FedEx to Monsanto Company (St. Louis, MO) for residue analysis. Samples were maintained frozen (~-20 °C) at the analytical laboratory. In preparation for analysis, samples (except oil) were milled in a cryomill, which pulverizes samples into a fine powder at cryogenic temperatures.

The petitioner submitted adequate descriptions of the processing procedures including material balance summaries. The cotton processing procedure is summarized below in Figure 1, which was copied without alteration from MRID 48728703.

FIGURE 1. Processing Flowchart for Cotton.



B.3. Analytical Methodology

Samples were analyzed for residues of dicamba and the metabolites DCGA, DCSA, and 5-hydoxy dicamba using a LC/MS/MS method from Monsanto Company: Analytical Method AG-ME-1381-01. For a complete description of the LC/MS/MS method, refer to the 860.1340 DER for MRID 48728702. We note that the analytical method does not specify conversion of metabolite residues to parent equivalents; therefore, quantifiable residues of 5-OH dicamba, DCSA, and DCGA were converted to parent equivalents by the study reviewer using molecular weight conversion factors of 0.933, 1.068, and 0.991, respectively.

Briefly, samples were extracted with acetonitrile:water (40:60, v:v). An aliquot of the extract was hydrolyzed in 1 N HCl at 95 °C in an oven. The hydrolysate was partitioned with ethyl acetate:isooctane (20:80, v:v). Water was added to the organic phase and the sample was concentrated by evaporation until only the aqueous solution remained. The samples were acidified and analyzed using LC/MS/MS.

The LOQ, determined as the LLMV, was 0.02 ppm for each analyte in each cotton matrix. The 0.020 ppm fortification level was used to statistically determine the LODs and LOQs for the four analytes of the method in undelinted cotton seed (RAC). The LODs for the analytes in undelinted cotton seed were 0.0035, 0.0041, 0.0057 and 0.0089 ppm for DCSA, DCGA, 5-hydroxydicamba and dicamba, respectively. The LOQs for the analytes in undelinted cotton seed were 0.0105, 0.0121, 0.0171 and 0.0266 ppm for DCSA, DCGA, 5-hydroxydicamba and dicamba, respectively (reported as analyte found per se). The LODs and LOQs were not statistically determined in the processed fractions.

The analytical method was validated prior to and in conjunction with the analysis of the submitted samples.

C. RESULTS AND DISCUSSION

Sample storage conditions and durations for samples of cotton and processed commodities are reported in Table C.2. Samples were stored under frozen (-20 °C) at the analytical laboratory from harvest/processing to analysis. Processing took place within 118-169 days of harvest. Sample analyses were completed within 152-196 days (5.0-6.4 months) for cotton (RAC) and 19-40 days (0.6-1.3 months) for processed commodities. All samples were analyzed on the day of extraction. A storage stability study for dicamba, DCGA, and DCSA on cotton was submitted in conjunction with the field trial study (refer to the 860.1380 der for MRID 48728704). Residues of dicamba, DCGA, and DCSA were stable during freezer storage for up to 277 days (9 months) in undelinted cotton seed. The stability of 5-OH dicamba was not determined concurrently because it was not found in undelinted cotton seed. These data are acceptable to support the storage conditions and durations of the undelinted cotton seed samples from the submitted field trials. Storage stability data for the processed commodities was not provided; however, storage stability data is not required as the processed commodities were generally analyzed within 30 days of processing.

Method validation and concurrent method recovery data for the LC/MS/MS method are presented in Table C.1. The LC/MS/MS method used to analyze cotton samples for residues of dicamba, 5-OH dicamba, DCGA, and DCSA was adequate for data collection based on acceptable method and concurrent method recovery data. Concurrent recoveries were generally within the acceptable range of 70-120% in cotton and processed commodity samples fortified with dicamba, 5-OH dicamba, DCGA, and DCSA each at 0.02-0.200 ppm. The fortification levels were adequate to bracket residues found in treated samples. Apparent residues of dicamba, 5-OH dicamba, DCGA, DSGA were below the LOQ (<0.02 ppm) in/on all samples of untreated undelinted cotton seed (RAC), hulls, meal, alkali refined oil, and RBD oil. Concurrent recoveries were corrected for apparent for residues in controls; residues in treated samples were not corrected for residues in controls.

Residue data from the cotton processing study are reported in Table C.3. Following three or four applications of the 4 lb ae/gal SC formulation at a total rate of 2 lb ae/A, using PHIs of 71-79 days and 6-7 days, respectively, the combined residues of dicamba, 5-OH dicamba, DCGA, and DCSA (in parent equivalents) were below the LOQ (<0.08)-<0.78 ppm in/on undelinted cotton seed (RAC), <0.081-<0.79 ppm in hulls, below the LOQ (<0.08)-<0.28 ppm in meal, and below the LOQ (<0.08 ppm) in alkali refined oil and RBD oil.

The processing data indicate that the combined residues do not concentrate in hulls, meal, alkali refined oil, or RBD oil (average processing factors of 0.86x, 0.49, 0.11x, and 0.11x, respectively).

The processing factors calculated in this study for combined residues of dicamba, 5-OH dicamba, DCGA, and DCSA in cotton were less than the theoretical concentration factor of 3.8x for hulls, 2.2x for meal, and 6.3x for oil (based separation of components; OPPTS 860.1520, Table 3).

TABLE C.1. Summary of Method and Concurrent Recoveries of Dicamba and its Metabolites from Cotton Matrices

Matrix	Analyte	Spike Level (ppm)	Sample Size (n)	Recoveries (%)[1]	Mean ± Std. Dev. (%)[2]
Method Validation Recoveries					
Undelinted Cotton seed (RAC)[3]	Dicamba	0.005	5	187, 73, 172, 105, 102	128 ± 49
		0.010	5	129, 149, 93, 78, 125	115 ± 29
		0.020	5	119, 100, 123, 101, 124	113 ± 12
		0.200	5	94, 96, 95, 97, 100	96.1 ± 2.2
		10.0	5	68, 91, 91, 100, 98	89.6 ± 13

TABLE C.1. Summary of Method and Concurrent Recoveries of Dicamba and its Metabolites from Cotton Matrices

Matrix	Analyte	Spike Level (ppm)	Sample Size (n)	Recoveries (%)[1]	Mean ± Std. Dev. (%)[2]
Undelinted Cotton seed (continued)	5-OH Dicamba	0.005	5	68, 56, 101, 84, 105	82.9 ± 21
		0.010	5	106, 97, 76, 110, 82	94.1 ± 15
		0.020	5	96, 98, 115, 117, 114	108 ± 10
		0.200	5	98, 104, 105, 104, 101	102 ± 3.0
		10.0	5	69, 90, 79, 93, 104	87.1 ± 13
	DCGA	0.005	5	140, 115, 88, 98, 129	114 ± 22
		0.010	5	90, 108, 96, 79, 104	95.6 ± 11
		0.020	5	81, 98, 93, 77, 86	86.9 ± 8.5
		0.200	5	90, 87, 88, 84, 96	88.9 ± 4.3
		10.0	5	73, 96, 89, 94, 90	88.6 ± 8.9
	DCSA	0.005	5	88, 95, 90, 134, 98	101 ± 19
		0.010	5	70, 84, 102, 96, 98	89.8 ± 13
		0.020	5	100, 82, 97, 97, 95	94.0 ± 6.9
		0.200	5	95, 100, 105, 96, 101	98.9 ± 4.0
		10.0	5	81, 92, 95, 93, 97	91.6 ± 6.4
Meal	Dicamba	0.020	5	102, 101, 98, 111, 108	104 ± 5.3
		0.200	5	97, 99, 96, 100, 94	96.9 ± 2.4
	5-OH Dicamba	0.020	5	72, 108, 92, 77, 92	88.1 ± 14
		0.200	5	98, 94, 88, 99, 90	93.4 ± 4.9
	DCGA	0.020	5	99, 108, 102, 103, 102	102 ± 3.3
		0.200	5	88, 86, 85, 88, 85	86.0 ± 1.5
	DCSA	0.020	5	101, 96, 100, 90, 98	96.5 ± 4.4
		0.200	5	82, 84, 86, 91, 89	86.2 ± 3.8
Hulls	Dicamba	0.020	5	97, 100, 106, 99, 99	100 ± 3.4
		0.200	5	97, 101, 102, 100, 99	100 ± 2.1
	5-OH Dicamba	0.020	5	87, 78, 106, 89, 97	91.2 ± 10
		0.200	5	114, 107, 117, 99, 105	108 ± 7.4
	DCGA	0.020	5	116, 117, 115, 121, 112	116 ± 3.4
		0.200	5	89, 86, 86, 84, 82	85.2 ± 2.4
	DCSA	0.020	5	109, 102, 94, 97, 96	99.5 ± 6.1
		0.200	5	96, 102, 92, 100, 99	97.4 ± 3.9
Alkali Refined Oil	Dicamba	0.020	5	99, 96, 97, 99, 98	97.6 ± 1.2
		0.200	5	99, 100, 105, 99, 97	100 ± 2.8
	5-OH Dicamba	0.020	5	95, 101, 79, 92, 88	90.8 ± 8.0
		0.200	5	110, 103, 88, 110, 100	102 ± 9.0
	DCGA	0.020	5	100, 102, 99, 103, 113	103 ± 5.7
		0.200	5	97, 89, 92, 92, 91	91.8 ± 2.8
	DCSA	0.020	5	102, 93, 90, 93, 100	95.2 ± 5.1
		0.200	5	103, 106, 100, 104, 105	103 ± 2.1

TABLE C.1. Summary of Method and Concurrent Recoveries of Dicamba and its Metabolites from Cotton Matrices

Matrix	Analyte	Spike Level (ppm)	Sample Size (n)	Recoveries (%)[1]	Mean ± Std. Dev. (%)[2]
Concurrent Recoveries					
Undelinted Cotton seed	Dicamba	0.02	3	78.1, 118, 105	100 ± 20
		0.20	3	91.9, 103, 108	101 ± 8.3
	5-OH Dicamba	0.02	2	85.5, 96.5	91.0
		0.20	2	90.0, 89.5	89.8
	DCGA	0.02	2	108, 119	113
		0.20	2	109, 114	112
	DCSA	0.02	2	84.9, 100	92.4
		0.20	2	96.4, 98.7	97.6
RBD Oil	Dicamba	0.02	2	115, 110	112
		0.20	2	106, 105	105
	5-OH Dicamba	0.02	2	119, 101	110
		0.20	2	98.5, 108	103
	DCGA	0.02	2	112, 106	109
		0.20	2	97.0, 89.5	93.3
	DCSA	0.02	2	108, 123	115
		0.20	2	104, 101	103
Alkali Refined Oil	Dicamba	0.02	2	103, 109	106
		0.20	2	101, 104	102
	5-OH Dicamba	0.02	2	94.5, 75.5	85.0
		0.20	2	92.0, 114	103
	DCGA	0.02	2	120, 119	120
		0.20	2	99.5, 99.0	99.3
	DCSA	0.02	2	113, 97.5	105
		0.20	2	109, 105	107
Hulls	Dicamba	0.02	3	109, 120, 101	110 ± 9.3
		0.20	3	79.0, 107, 103	96.4 ± 15
	5-OH Dicamba	0.02	2	87.5, 99.5	93.5
		0.20	2	84.5, 91.5	88.0
	DCGA	0.02	2	112, 106	109
		0.20	2	106, 104	105
	DCSA	0.02	2	95.9, 96.4	96.1
		0.20	2	98.1, 97.2	97.6
Meal	Dicamba	0.02	2	98.8, 114	106
		0.20	2	101, 103	102
	5-OH Dicamba	0.02	2	100, 79.0	89.5
		0.20	2	91.5, 92.5	92.0
	DCGA	0.02	2	104, 104	104
		0.20	2	111, 109	110
	DCSA	0.02	2	79.8, 87.4	83.6
		0.20	2	94.1, 105	99.8

[1] The concurrent recoveries were corrected for apparent residues in the unfortified control samples.
[2] Standard deviation is not applicable for sample sizes n < 3 samples.

[3] Recoveries for method validation undelinted cotton seed are also presented in the crop field trial DER (refer to 48728703.DER1).

TABLE C.2. Summary of Storage Conditions.

Matrix	Analyte	Storage Temperature	Actual Storage Duration[1]	Limit of Demonstrated Storage Stability[2]
Dicamba				
Undelinted Cotton Seed	Dicamba	Ambient at field sites; -12 °C at processor; -20 °C at analytical laboratory	152-196 days (5.0-6.4 months)	Residues of dicamba are stable during freezer storage for up to 277 days (9 months) in undelinted cotton seed.
Hulls			33-40 days (1.1-1.3 months)	Storage stability data for the processed fractions was not provided. However, storage stability data is not required as the processed fractions were generally analyzed within 30 days of processing.
Meal			26-27 days (0.9 months)	
Alkali Refined Oil			24-25 days (0.8 months)	
RBD Oil			19-20 days (0.6-0.7 months)	
Dicamba Metabolites				
Undelinted Cotton Seed	5-OH Dicamba, DCGA, DCSA	Ambient at field sites; -12 °C at processor; -20 °C at analytical laboratory	152-189 days (5.0-6.2 months)	Residues of dicamba, DCGA, and DCSA are stable during freezer storage for up to 277 days (9 months) in undelinted cotton seed. The stability of 5-OH dicamba was not determined concurrently because it was not found in undelinted cotton seed.
Hulls			33-34 days (1.1 months)	Storage stability data for the processed fractions was not provided. However, storage stability data is not required as the processed fractions were generally analyzed within 30 days of processing.
Meal			26-27 days (0.9 months)	
Alkali Refined Oil			24-25 days (0.8 months)	
RBD Oil			19-20 days (0.6-0.7 months)	

[1] Interval from harvest/processing to analysis; all samples were analyzed on the day of extraction. The samples were processed within 118-169 days of harvest.
[2] Refer to the 860.1380 der for MRID 48728704.

TABLE C.3. Residue Data for Dicamba and Metabolites from Cotton Processing Studies[1]

RAC	Processed Commodity	Total Rate lb ae/A (kg ae/A)	TRT[2]	PHI (days)	Residues (ppm)[3]					Processing Factor[4,5]				
					Dicamba	5-OH Dicamba	DCGA	DCSA	Combined Residues	Dicamba	5-OH Dicamba	DCGA	DCSA	Combined Residues
Fisk, Butler, MO; 2010 (MO1)														
Cotton	Undelinted Seed	2.0 (2.2)	2	79	<0.02	<0.02	<0.02	<0.02	<0.08	--	--	--	--	--
	Hulls				<0.02	<0.02	0.02	0.02	<0.08	NC[5]	NC	>1.0x	1.0x	1.1x
	Meal				<0.02	<0.02	<0.02	<0.02	<0.08	NC	NC	NC	NC	NC
	Alkali Refined Oil				<0.02	<0.02	<0.02	<0.02	<0.08	NC	NC	NC	NC	NC
	RBD Oil				<0.02	<0.02	<0.02	<0.02	<0.08	NC	NC	NC	NC	NC
Cotton	Undelinted Seed	2.0 (2.2)	4	7	0.70	<0.02	0.04	0.03	<0.78	--	--	--	--	--
	Hulls				0.70	<0.02	0.05	0.03	<0.79	1.0x	NC	1.3x	1.0x	1.0x
	Meal				0.22	<0.02	<0.02	<0.02	<0.28	0.31x	NC	0.50x	<0.67x	0.35x
	Alkali Refined Oil				<0.02	<0.02	<0.02	<0.02	<0.08	<0.03x	NC	<0.50x	<0.67x	0.10x
	RBD Oil				<0.02	<0.02	<0.02	<0.02	<0.08	<0.03x	NC	<0.50x	<0.67x	0.10x
Uvalde, Uvalde, TX; 2010 (TX4)														
Cotton	Undelinted Seed	2.0 (2.2)	2	71	<0.02	<0.02	<0.02	<0.02	<0.08	--	--	--	--	--
	Hulls				<0.02	<0.02	<0.02	0.02	<0.08	NC	NC	NC	>1.0x	1.0x
	Meal				<0.02	<0.02	<0.02	<0.02	<0.08	NC	NC	NC	>1.0x	1.0x
	Alkali Refined Oil				<0.02	<0.02	<0.02	<0.02	<0.08	NC	NC	NC	NC	NC
	RBD Oil				<0.02	<0.02	<0.02	<0.02	<0.08	NC	NC	NC	NC	NC
Cotton	Undelinted Seed	2.0 (2.2)	4	6	0.60	<0.02	0.04	0.05	<0.69	--	--	--	--	--
	Hulls				0.16	<0.02	0.08	0.06	<0.26	0.27x	NC	2.0x	1.2x	0.37x
	Meal				<0.02	<0.02	<0.02	<0.02	<0.08	<0.03x	NC	<0.50x	<0.40x	0.12x
	Alkali Refined Oil				<0.02	<0.02	<0.02	<0.02	<0.08	<0.03x	NC	<0.50x	<0.40x	0.12x
	RBD Oil				<0.02	<0.02	<0.02	<0.02	<0.08	<0.03x	NC	<0.50x	<0.40x	0.12x

TABLE C.3. Residue Data for Dicamba and Metabolites from Cotton Processing Studies[1]

RAC	Processed Commodity	Total Rate lb ae/A (kg ae/A)	TRT[2]	PHI (days)	Residues (ppm)[3]					Processing Factor[4,5]				
					Dicamba	5-OH Dicamba	DCGA	DCSA	Combined Residues	Dicamba	5-OH Dicamba	DCGA	DCSA	Combined Residues
Average														
Cotton	Undelinted Seed	2.0 (2.2)	2	71-79						--	--	--	--	--
	Hulls									NC	NC	1.0x	1.0x	1.0x
	Meal									NC	NC	NC	1.0x	1.0x
	Alkali Refined Oil									NC	NC	NC	NC	NC
	RBD Oil									NC	NC	NC	NC	NC
Cotton	Undelinted Seed	2.0 (2.2)	4	6-7						--	--	--	--	--
	Hulls									0.63x	NC	1.6x	1.1x	0.69x
	Meal									0.17x	NC	0.50x	0.53x	0.23x
	Alkali Refined Oil									0.03x	NC	0.50x	0.53x	0.11x
	RBD Oil									0.03x	NC	0.50x	0.53x	0.11x
Cotton	Undelinted Seed	2.0 (2.2)	2-4	6-79						--	--	--	--	--
	Hulls									0.63x	NC	1.4x	1.1x	0.86x
	Meal									0.17x	NC	0.50x	0.69x	0.49x
	Alkali Refined Oil									0.03x	NC	0.50x	0.53x	0.11x
	RBD Oil									0.03x	NC	0.50x	0.53x	0.11x

[1] Each trial site was planted with Event MON 88701 cotton that is tolerant to dicamba herbicide and glufosinate-ammonium herbicide.
[2] TRT 2: Applications made at preemergence, the 6-leaf stage, and at the first white flower + 15 days stage; EP: Clarity. TRT 4: applications made at the 6-leaf stage, the first white flower + 15-days stage, the first open boll stage, and at 7 days prior to harvest; EP: Clarity.
[3] The LOQs were 0.02 ppm per analyte per matrix. Residues for individual analytes are presented per se and reflect the raw data. Combined residues were calculated by the study reviewer and are expressed in terms of parent equivalents. Quantifiable residues of 5-OH dicamba, DCSA, and DCGA were converted to parent equivalents by the study reviewer using molecular weight conversion factors of 0.933, 1.068, and 0.991, respectively. When calculating combined residues, the LOQ was used for values reported as ND or between the LOD and LOQ.
[4] Processing Factor = [Measured residue for analyte in the processed fraction] / [Measured residue for analyte in the RAC].
[5] NC = Not calculated; residues were below the LOQ in the RAC and processed fraction.

D. CONCLUSION

The submitted cotton processing study is acceptable. Following three or four applications of the 4 lb ae/gal SC formulation at a total rate of 2 lb ae/A, using PHIs of 71-79 days and 6-7 days, respectively, the combined residues of dicamba, 5-OH dicamba, DCGA, and DCSA (in parent equivalents) were below the LOQ (<0.08)-<0.78 ppm in/on undelinted cotton seed (RAC), <0.081-<0.79 ppm in hulls, below the LOQ (<0.08)-<0.28 ppm in meal, and below the LOQ (<0.08 ppm) in alkali refined oil and RBD oil. The processing data indicate that the combined residues do not concentrate in hulls, meal, alkali refined oil, or RBD oil (average processing factors of 0.86x, 0.49, 0.11x, and 0.11x, respectively). The observed concentration factors were less than the theoretical concentration factors.

Samples were analyzed using acceptable methods, and the study is supported by adequate storage stability data.

E. REFERENCES

None.

F. DOCUMENT TRACKING

Petition Number(s): 2F8067
DP Barcode(s): 408384
PC Code: 029801, 029802, 029806, 128931, 128944, & 129043

Template Version June 2005

Primary Evaluator		Date: 11/15/2012
	Versar, Inc.	
Approved by	Peter Savoia	Date: 04/22/2013
	Peter Savoia, Chemist, OCSPP-HED-RAB V/VII	

Note: This Data Evaluation Record (DER) was originally prepared under contract by Versar, Inc. (6850 Versar Center, Springfield, VA 22151; submitted 11/15/12). The DER has been reviewed by HED and revised to reflect current Office of Pesticide Programs (OPP) policies.

STUDY REPORT:

48728704. Maher, D.; Foster, J. (2012) Determination of the Stability of Dicamba Residues in Dicamba-Tolerant Cotton under Frozen Storage Conditions. Project Number: REG/10/504/OCR, MSL0023058. Unpublished study prepared by Monsanto Company. 144p.

EXECUTIVE SUMMARY:

Monsanto has submitted the results of a storage stability study with field-incurred residues of dicamba and its metabolites 3,6-dichlorosalicylic acid (DCSA) and 3,6-dichlorogentisic acid (DCGA) in/on dicamba-tolerant cotton identified as Event MON 88701 (formerly GH_S26695). Event MON 88701 cotton expresses a modified dicamba mono-oxygenase (DMO) gene derived from *Stenotrophomonas maltophilia* and the bialaphos resistance (BAR) gene isolated from *Streptomyces hygroscopicus*. The expression of DMO confers dicamba tolerance and the expression of BAR confers glufosinate tolerance to Event MON 88701 cotton. Samples of undelinted cotton seeds were obtained from a crop field trial conducted by Monsanto (MRID 48728703). Samples were collected 6 days following the last of four post-emergence broadcast applications of a 4 lb ae/gal water soluble concentrate (SL) formulation of dicamba diglycolamine salt (Clarity Herbicide; MON 54140) at 0.5 lb ae/A (0.56 kg ae/ha), for a total application rate of 2.0 lb ae/A (2.2 kg ae/ha). The applications were made at the 6-leaf stage, first white flower +15 days stage, first open boll stage and 7-days preharvest. Samples were placed in frozen storage (≤-10 °C) from the time of initial sampling/milling until analysis at ~0, 1, 2, 4, 6 and 9 months from the initiation of the storage stability study.

Undelinted cotton seed samples were analyzed for residues of dicamba, DCSA and DCGA using liquid chromatography with tandem mass spectrometry detection (LC/MS/MS), Monsanto method AG-ME-1381. The validated limit of quantitation (LOQ; determined as the lowest level of method validation, LLMV) was 0.02 ppm for dicamba, DCSA and DCGA. The method was acceptable for data collection based on adequate method validation and concurrent recovery data.

The results of the study indicate that field-incurred residues of dicamba and metabolites DCSA and DCGA are relatively stable in/on samples of undelinted cotton seed stored frozen at ≤-10 °C for up to 9 months. Corrected mean recoveries at 9 months (calculated as percentage of the 0-month results) were 76%, 117% and 95%, respectively, for dicamba, DCGA and DCSA.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the storage stability data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, D408384.

COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance and No Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Dicamba (3,6-dichloro-2-methoxybenzoic acid) is a selective benzoic acid herbicide registered for the control of weeds prior to their emergence. The Dicamba Reregistration Eligibility Decision (RED) was issued December 2005. The chemical structure and nomenclature of dicamba and its metabolites DSCA and DCGA are presented in Table A.1. The physicochemical properties of the technical grade of dicamba acid are presented in Table A.2.

TABLE A.1. Test Compound Nomenclature.

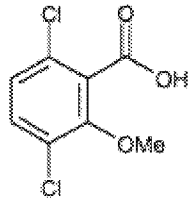
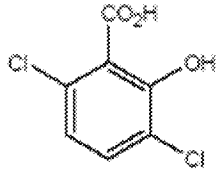
Compound	
Common name	Dicamba
Company experimental name	MON 11900
IUPAC name	3,6-dichloro-o-anisic acid or 3,6-dichloro-2-methoxybenzoic acid
CAS name	3,6-dichloro-2-methoxybenzoic acid
CAS registry number	1918-00-9 (dicamba acid), 104040-79-1 (diglycolamine salt), or 53404-28-7 (monoethanolamine salt)
End-use product	Clarity® Herbicide: SL formulation containing 4 lb ae/gal
Compound	
Common name	DCSA
Company experimental name	3,6-dichlorosalicylic acid; MON 52708
IUPAC/CAS name	3,6-dichloro-2-hydroxybenzoic acid
CAS registry number	3401-80-7

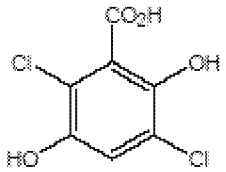
TABLE A.1. Test Compound Nomenclature.	
Compound	
Common name	DCGA
Company experimental name	3,6-dichlorogentisic acid (DCGA); MON 52724
IUPAC/CAS name	2,5-dichloro-3,6-dihydroxybenzoic acid
CAS registry number	18688-01-2

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound: Dicamba.		
Parameter	Value	Reference
Melting point	114-116 EC (PAI) 90-100 EC (87% TGAI)	Residue Chemistry Chapter of the Dicamba RED, DP# 317699, 12/20/05, C. Olinger
pH	2.5-3.0 (87% TGAI)	
Density	1.57 g/mL at 25 EC (87% TGAI)	
Water solubility	0.5 g/100 mL at 25 EC (PAI)	
Solvent solubility	g/100 mL at 25 EC (PAI) dioxane 118.0 ethanol 92.2 isopropyl alcohol 76.0 methylene chloride 26.0 acetone 17.0 toluene 13.0 xylene 7.8 heavy aromatic naphthalene 5.2	
Vapor pressure	3.4 x 10^{-5} mm Hg at 25 EC (PAI)	
Dissociation constant, pK_a	1.97 (PAI)	
Octanol/water partition coefficient, Log(K_{OW})	0.1 (PAI)	
UV/visible absorption spectrum	neutral: 511 (275 nm) acidic (pH 0-1): 1053 (281 nm) basic (pH 13-14): 469 (274 nm)	

B. EXPERIMENTAL DESIGN

B.1. Sample Handling and Preparation

The storage stability study was conducted by Monsanto Company at its Environmental Sciences Technology Center in St. Louis, Missouri.

Undelinted cotton seed samples were obtained from a crop field trial study conducted by Monsanto (MRID 48728703) using dicamba-tolerant cotton identified as Event MON 88701 (formerly GH_S26695). Event MON 88701 cotton expresses a modified dicamba mono-oxygenase (DMO) gene derived from *Stenotrophomonas maltophilia* and the bialaphos resistance (BAR) gene isolated from *Streptomyces hygroscopicus*. The expression of DMO confers dicamba tolerance and the expression of BAR confers glufosinate tolerance to Event MON 88701 cotton.

In one trial conducted in Hockley, TX (Site TX2), samples of undelinted cotton seeds were collected from treatment plot 4 (TRT 4) following the last of four post-emergence broadcast applications of a 4 lb ae/gal SL formulation of dicamba diglycolamine salt (Clarity Herbicide; MON 54140) at 0.5 lb ae/A (0.56 kg ae/ha), for a total application rate of 2.0 lb ae/A (2.2 kg ae/ha). The applications were made at the 6-leaf stage, first white flower +15 days stage, first open boll stage and 7-days preharvest using a backpack sprayer in spray volumes of ~20 gal/A (187 L/ha). A non-ionic surfactant (NIS) and ammonium sulfate (AMS) were added to the spray mixture.

Single composite cotton seed samples (~1 kg) were collected from the control and treated plot 6 days after the last application and ginned at the field site one day after harvest. Samples were stored at ambient temperature until ginning. The undelinted cotton seed samples were placed in frozen storage at the field trial site within 1 hour of sampling and shipped via ACDS freezer truck to Monsanto the next day (2 days after harvest). The samples were placed in frozen storage at Monsanto (-10 °C) and were removed from frozen storage and analyzed at intervals of ~0, 1, 2, 4, 6 and 9 months from the initiation of the storage stability study, which occurred 121 days after harvest. Actual storage durations were 0, 31, 60, 125, 194, and 277 days. The undelinted cotton seed subsample used for the storage stability study was prepared at Monsanto two days before the Day 0 analysis (119 days after harvest) using a cryomill, which pulverizes samples into a fine powder at cryogenic temperatures. Concurrent recovery samples were fortified at each interval at 0.04, 0.3 and 2.0 ppm, using a mixed standard containing dicamba and DCSA and a separate solution containing only DCGA.

A subsample of the treated undelinted cotton seed sample was originally milled 20 days after harvest and analyzed 38 days after harvest, as part of the crop field trial study (see above). Due to an inadequate amount of milled material, new subsamples were milled 119 days after harvest for use in the storage stability study. Three replicates of the original milled subsample were re-analyzed shortly after the initiation of the storage stability study to assess stability of residues during this period. The results of the original and repeat analyses were similar. The Study Author attributed differences between residues in the original analysis for the crop field trial study and the Day 0 analysis for the storage stability study to variability between subsamples.

B.2. Analytical Methodology

Samples were analyzed for residues of dicamba and the metabolites DCGA and DCSA using a LC/MS/MS method from Monsanto: Analytical Method AG-ME-1381-01. For a complete description of the LC/MS/MS method, refer to the 860.1340 DER for MRID 48728702.

Briefly, samples were extracted with acetonitrile:water (40:60, v:v). An aliquot of the extract was hydrolyzed in 1 N HCl at 95 °C in an oven. The hydrolysate was partitioned with ethyl acetate:isooctane (20:80, v:v). Water was added to the organic phase and the sample was concentrated by evaporation until only the aqueous solution remained. The samples were acidified and analyzed using LC/MS/MS.

The LOQ (determined as the LLMV) was 0.02 ppm for dicamba, DCSA and DCGA in undelinted cotton seed. We note that the petitioner also presented calculated values statistically determined for the LOD and LOQ based on recoveries obtained at the 0.020 ppm fortification level from the method validation study. The LODs were 0.0089, 0.0035 and 0.0041 ppm for dicamba, DCSA and DCGA, respectively. The LOQs were 0.0266, 0.0105 and 0.0121 ppm for dicamba, DCSA and DCGA, respectively.

The analytical method was validated prior to the storage stability study and in conjunction with analysis of frozen storage stability samples.

C. RESULTS AND DISCUSSION

Concurrent recovery data are presented in Table C.1. The method validation data are presented in the DER for MRID 48728703. The data indicate that the LC/MS/MS was adequate for determination of residues of dicamba, DCSA and DCGA in undelinted cotton seed. Concurrent recoveries were within the acceptable range of 70-120% for all analytes in samples fortified at 0.04, 0.30, and 2.0 ppm each. Apparent residues in control samples were less than the method LOQ (<0.02 ppm), and adequate example chromatograms were provided. Concurrent recoveries were corrected for residues found in the control samples.

The results of the storage stability study are presented in Table C.2. The study results indicate that residues of dicamba, DCSA and DCGA are stable in undelinted cotton seed stored frozen (≤-10 °C) for up to 9 months. Corrected mean recoveries were 76%, 117% and 95%, respectively, for dicamba, DCGA and DCSA in/on undelinted cotton seed at 9 months (calculated as percentage of the 0-month results).

A graph depicting the storage stability of field-incurred residues of dicamba and its metabolites DCSA and DCGA in/on undelinted cotton seed is presented in Figure C.1.

TABLE C.1 Summary of Concurrent Recoveries of Dicamba and its Metabolites from Undelinted Cotton Seed.

Matrix	Analyte	Storage Interval (Months)	Fortification Level (ppm)	Sample size (n)	Recoveries[1] (%)	Mean ± Std Dev.[2] (%)
Undelinted cotton seed	Dicamba	0	0.04	1	79.8	92.9 ± 11.5
			0.30	1	98.0	
			2.00	1	101	
		1	0.04	1	90.3	97.0 ± 7.39
			0.30	1	96.3	
			2.00	1	105	
		2	0.04	1	97.3	97.6 ± 1.77
			0.30	1	96.0	
			2.00	1	99.5	
		4	0.04	1	107	111 ± 4.00
			0.30	1	111	
			2.00	1	115	
		6	0.04	1	117	91.1 ± 22.3
			0.30	1	80.2	
			2.00	1	76.7	
		9	0.04	1	112	105 ± 6.26
			0.30	1	105	
			2.00	1	99.5	
	DCGA	0	0.04	1	103	105 ± 2.08
			0.30	1	107	
			2.00	1	104	
		1	0.04	1	83.5	84.7 ± 1.43
			0.30	1	84.4	
			2.00	1	86.3	
		2	0.04	1	96.7	100 ± 3.22
			0.30	1	101	
			2.00	1	103	
		4	0.04	1	104	103 ± 3.21
			0.30	1	99.0	
			2.00	1	105	
		6	0.04	1	94.0	93.4 ± 2.32
			0.30	1	95.3	
			2.00	1	90.8	
		9	0.04	1	95.0	105 ± 9.54
			0.30	1	114	
			2.00	1	106	
	DCSA	0	0.04	1	99.9	112 ± 11.3
			0.30	1	115	
			2.00	1	122	
		1	0.04	1	115	105 ± 12.3
			0.30	1	108	
			2.00	1	91.0	
		2	0.04	1	99.4	94.5 ± 4.25
			0.30	1	92.2	
			2.00	1	91.9	
		4	0.04	1	98.7	95.0 ± 6.29
			0.30	1	98.5	
			2.00	1	87.7	
		6	0.04	1	79.0	76.0 ± 4.71
			0.30	1	70.6	
			2.00	1	78.5	

TABLE C.1 Summary of Concurrent Recoveries of Dicamba and its Metabolites from Undelinted Cotton Seed.

Matrix	Analyte	Storage Interval (Months)	Fortification Level (ppm)	Sample size (n)	Recoveries[1] (%)	Mean ± Std Dev.[2] (%)
		9	0.04	1	98.5	97.7 ± 3.01
			0.30	1	94.2	
			2.00	1	100	

[1] Recovery values were corrected for background levels in controls.
[2] Standard deviations were determined by the study reviewer.

TABLE C.2 Stability of Field-Incurred Residues of Dicamba, DCGA and DCSA in Dicamba-Resistant Undelinted Cotton Seed Following Storage at -10°C

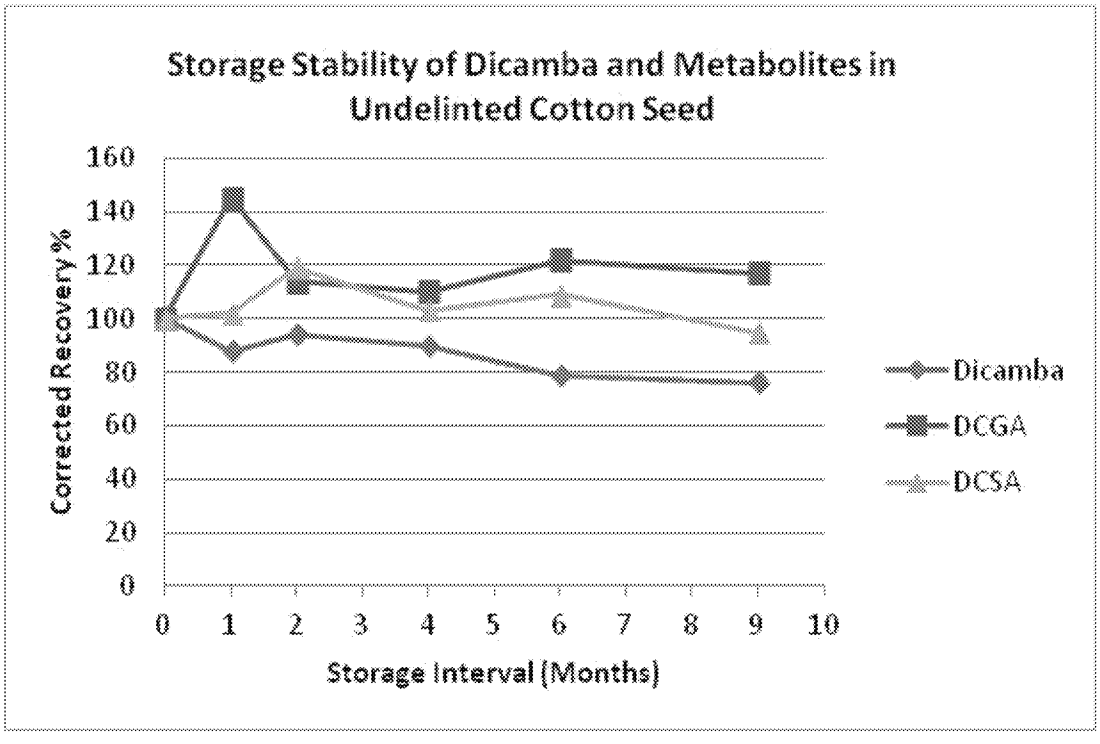
Commodity	Analyte	Spike Level (ppm)	Storage interval (months)	Recovered Residues (ppm)	Mean Recovered Residues (ppm)	Mean Recovery[1] (%)	Corrected Recovered Residues[2] (ppm)	Corrected Mean Recovery[3] (%)
Undelinted cotton seed	Dicamba	Incurred[4]	0	1.01, 0.91, 0.78, 0.83, 0.78, 0.82	0.85	100	0.91	--
			1	0.82, 0.76, 0.79, 0.75	0.78	92	0.80	88
			2	0.70, 0.89, 0.90, 0.88	0.84	99	0.86	94
			4	0.94, 0.82, 0.88, 1.00	0.91	107	0.82	90
			6	0.54, 0.62, 0.70, 0.72, 0.64, 0.72	0.66	78	0.72	79
			9	0.72, 0.70, 0.80, 0.75, 0.72, 0.71	0.73	86	0.70	76
	DCGA	Incurred[4]	0	0.13, 0.12, 0.12, 0.13, 0.11, 0.12	0.12	100	0.11	--
			1	0.14, 0.14, 0.16, 0.15	0.14	117	0.17	145
			2	0.12, 0.13, 0.13, 0.14	0.13	108	0.13	114
			4	0.14, 0.13, 0.12, 0.12	0.13	108	0.13	110
			6	0.13, 0.14, 0.15, 0.13, 0.12, 0.14	0.13	108	0.14	122
			9	0.14, 0.13, 0.14, 0.15, 0.14, 0.13	0.14	117	0.13	117
	DCSA	Incurred[4]	0	0.24, 0.24, 0.24, 0.22, 0.24, 0.23	0.23	100	0.21	--
			1	0.22, 0.21, 0.23, 0.24	0.22	96	0.21	102
			2	0.20, 0.24, 0.22, 0.25	0.23	100	0.24	119

TABLE C.2 Stability of Field-Incurred Residues of Dicamba, DCGA and DCSA in Dicamba-Resistant Undelinted Cotton Seed Following Storage at -10°C

Commodity	Analyte	Spike Level (ppm)	Storage interval (months)	Recovered Residues (ppm)	Mean Recovered Residues (ppm)	Mean Recovery[1] (%)	Corrected Recovered Residues[2] (ppm)	Corrected Mean Recovery[3] (%)
			4	0.21, 0.20, 0.19, 0.21	0.20	87	0.21	103
			6	0.12, 0.18, 0.18, 0.17, 0.18, 0.18	0.17	74	0.22	109
			9	0.20, 0.17, 0.18, 0.21, 0.18, 0.18	0.19	83	0.19	95

[1] Recoveries for stored samples calculated by Versar reviewer as percentage of 0-month results.
[2] Corrected by the study reviewer for mean concurrent recovery (Table C.1.)
[3] Calculated by the study reviewer as (ppm corrected recovered residues in sample ÷ ppm corrected recovered residues in 0-day sample).
[4] Residues incurred following four applications of MON 54140, a SL formulation of dicamba diglycolamine salt containing 4 lb ae/gal (480 g/L), to cotton at application rates of ~0.5 lb ae/A/application (0.56 kg ae/ha).

Figure C.1 Graph of Dicamba Storage Stability in Undelinted Cotton Seed



D. CONCLUSION

The storage stability data are adequate and demonstrate the stability of field-incurred residues of dicamba and metabolites DCSA and DCGA in/on samples of undelinted cotton seed stored frozen for up to 9 months at ≤-10 °C. An acceptable method was used for quantitation of residues.

E. REFERENCES

Residue Chemistry Chapter of the Dicamba RED, DP# 317699, 12/20/05, C. Olinger

F. DOCUMENT TRACKING

Petition Number(s): 2F8067
DP Barcode(s): 408384
PC Code: 029801, 029802, 029806, 128931, 128944, & 129043